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HiSeq® 2500 System User Guide

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ILLUMINA PROPRIETARY
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Revision History

Part #	Rev.	Date	Description of Change
15035786	D	November 2014	Updated Rapid Run mode workflow for compatibility with HiSeq Rapid v2 chemistry. Replaced NaOH maintenance wash with Tween 20 and ProClin 300 maintenance wash including information about preparing, storing, and disposing of maintenance wash solution. Updated descriptions of maintenance wash and water wash to specify that a water wash is required after a run. Added workflow, input and output files, error handling, and quality scoring descriptions to Real-Time Analysis chapter. Updated VWR catalog # for alcohol wipes to 95041-714. Updated URL for Safety Data Sheets (SDS) to support.illumina.com/sds.html.
15035786	C	April 2014	Updated software descriptions to HiSeq Control Software v2.2, which includes the HiSeq v4 high output mode, removal of the control lane option, default Q-score binning, and the option to use different indexing schemes in each lane. Added the HiSeq v4 workflow for use with HiSeq v4 chemistry. Added calculation for total SBS priming volume.
15035786	B	November 2013	Removed reagent preparation instructions. For reagent prep instructions including information about various sequencing primers, see the documentation for the associated kit. Replaced the following reagents: • RMR for RMX
15035786	A	October 2012	Initial release.

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Overview

Chapter 1

Introduction

The HiSeq® system combines innovative engineering with proven SBS technology to set new standards in output, simplicity, and cost-effectiveness.

The HiSeq 2500 includes the following features:

- **Dual-surface imaging**—The HiSeq 2500 uses a 4-camera epifluorescence system with cutting-edge scanning technology to enable dual surface imaging.
- **Dual flow cells**—The HiSeq 2500 is a dual flow cell system, which allows sequencing of a single flow cell or 2 flow cells with different read lengths simultaneously.
- **On-instrument cluster generation**—The HiSeq 2500 provides the option of Rapid Run mode, which includes on-instrument cluster generation.
- **High-capacity reagent chiller**—The reagent compartment is a high-capacity chiller that holds enough reagents for the entire sequencing run.
- **Integrated fluidics for paired-end runs**—Integrated paired-end fluidics provide reagents from the reagent compartment to the flow cell for Read 2 resynthesis and for indexed sequencing.
- **Interface control options**—The instrument software interface provides options for setting up a run and operating the instrument using the touch screen monitor or the integrated keyboard.
- **Real-time base calling**—The instrument software extracts intensities from images and performs quality-scored base calling on the instrument computer, which allows monitoring of quality metrics during the run and saves time during subsequent data analysis.

  Downstream analysis of sequencing data can be performed with Illumina analysis software or third-party software on IlluminaCompute, Illumina BaseSpace, or a custom infrastructure.
- **BaseSpace connectivity**—The HiSeq 2500 features an option to send instrument health and sequencing data to the BaseSpace genomics cloud solution in real time to streamline instrument quality control and analysis.

HiSeq 2500 Components

The HiSeq 2500 system comprises the instrument, monitor, instrument control computer, and accessories, such as a keyboard, mouse, and barcode scanner. The instrument includes 4 main compartments: the optics module, flow cell compartment, fluidics compartment, and reagents compartment. Instrument operating status is indicated on an illuminated status bar.

Figure 1 External Components

A **Optics module**—Contains optical components that enable dual surface imaging of the flow cell, imaging A, C, G, and T at the same time using epifluorescence. The excitation laser beam passes through the objective and the fluorescence is collected through the same objective.

B **Flow cell compartment and library loading station**—Contains the vacuum-controlled flow cell stage, which holds the flow cell in place during sequencing runs. Using Rapid Run mode, the loading station transfers libraries to the flow cell for on-instrument cluster generation.

C **Fluidics compartment**—Contains fluidics pumps that deliver reagents to the flow cell, and then to the waste container.

D **Status bar**—Uses 3 colors to indicate instrument status. Blue indicates that the instrument is running, orange indicates that the instrument needs attention, and green indicates that the instrument is ready to begin the next run.

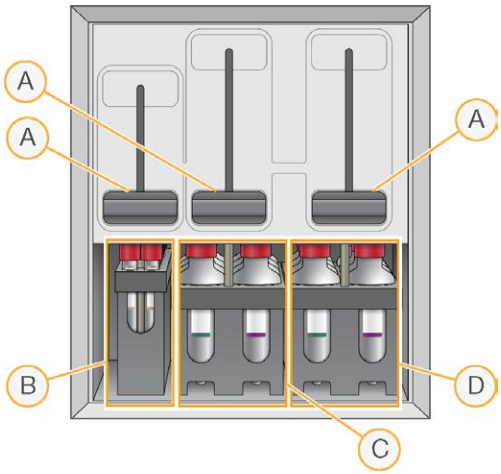
E **Reagent compartment**—Contains reagent racks that hold reagents for sequencing runs and wash solution for instrument washes.

Reagent Compartment

The reagent compartment is a high-capacity reagent chiller that holds 3 reagent racks: 2 for SBS reagents and 1 for clustering, indexing, and paired-end reagents. Sipper handles lower the sippers into the reagent bottles.

- **SBS reagent racks**—Hold 250 ml conical bottles. The reagent rack for flow cell A is located in the center position, and the rack for flow cell B is located in the far right position. Each reagent rack has numbered positions that correspond to connections on an internal reagent selector valve.
- **Clustering, indexing, and paired-end reagent rack**—Located to the left of racks A and B. It has 2 rows of numbered positions that hold 15 ml conical tubes containing cluster and paired-end reagents and indexing reagents. The left row is for flow cell A, and the right row is for flow cell B.
- **Reagent chiller**—The reagent chiller houses the reagent racks and maintains an internal temperature of 2°C to 8°C.

Figure 2 Reagent Compartment

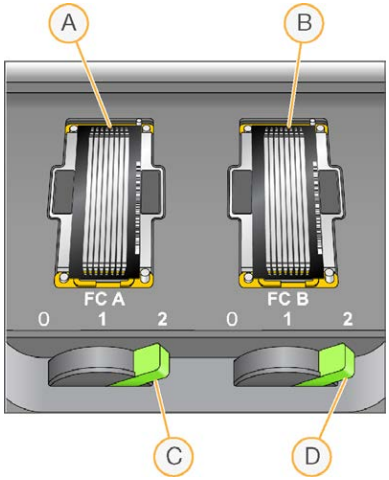


A Sipper handles
B Reagent rack for clustering, indexing, and paired-end reagents
C Reagent rack for SBS reagents for flow cell A
D Reagent rack for SBS reagents for flow cell B

Flow Cell Compartment

The flow cell compartment houses the flow cell stage, the thermal stations, the vacuum system, and the fluidics connections to each flow cell.

Figure 3 Flow Cell Stage With 2 Flow Cells



A Flow cell A
B Flow cell B
C Flow cell lever A
D Flow cell lever B

The flow cell on the left is flow cell A, and the flow cell on the right is flow cell B.

Each flow cell is seated on the flow cell stage, which moves in and out of the optics module as directed by the control software. The flow cell stage must be in the forward-most position to open the flow cell compartment door and load or remove a flow cell.

The flow cell is positioned on the flow cell holder with the inlet and outlet ports facing down, and held in place by a vacuum beneath each flow cell holder. The illuminated flow cell lever in front of each flow cell holder controls the vacuum. The flow cell lever turns green when the vacuum seal is secure.

Start the HiSeq 2500

1. Start the instrument control computer.
2. Log on to the operating system using the default user name and password.
   - User name: sbsuser
   - Password: sbs123

   Wait until it has loaded. If the default values do not work, consult your facility administrator for the site-specific user name and password.
3. Turn on the main power switch to the ON position. When facing the front of the instrument, the power switch is on the left side.
4. Wait at least 1 minute for the instrument devices to be properly configured and for the instrument drive called DoNotEject to initialize. A window opens when the drive is initialized. Close the window. If the window does not open, use MyComputer to check for the DoNotEject drive.

   NOTE
   Never eject the DoNotEject flash drive located inside the instrument chassis, or modify the files on it. This drive contains hardware configuration files and initializes whenever the instrument is turned on.
5. To ensure adequate disk space, archive the data on the instrument computer from previous runs to a network location.
6. Open the HiSeq Control Software (HCS) using the shortcut icon on the computer desktop. The control software takes a few minutes to initialize. When the software has initialized, the Mode Select screen opens and the initialization icon 

appears on the bottom-right corner of the screen.

Instrument and Control Computer Best Practices

- Do not turn on the computer while the instrument is running. Always turn on the computer before turning on the instrument.
- Do not turn off the instrument while the instrument control software is running.
- Wait 1 minute after turning off the instrument before turning it on again.
- Connect the USB cables for the instrument, the monitor, and the keyboard to the back of the computer before turning on the computer.
- Connect the barcode scanner and mouse to the USB ports on the front of the computer.

HiSeq 2500 Software

Three software applications are installed on the instrument computer:

- **HiSeq 2500 control software**—The HiSeq Control Software (HCS) interface guides you through the steps to set up a sequencing run. During the run, the control software operates instrument hardware, controls fluidics, sets temperatures, and provides a visual summary of quality statistics.
- **Real-Time Analysis software**—Integrated with the control software, Real-Time Analysis (RTA) performs base calling and assigns a quality score to each base for each cycle. For more information, see *Real-Time Analysis* on page 111.
- **Sequencing Analysis Viewer software**—Sequencing Analysis Viewer (SAV) provides detailed quality statistics.

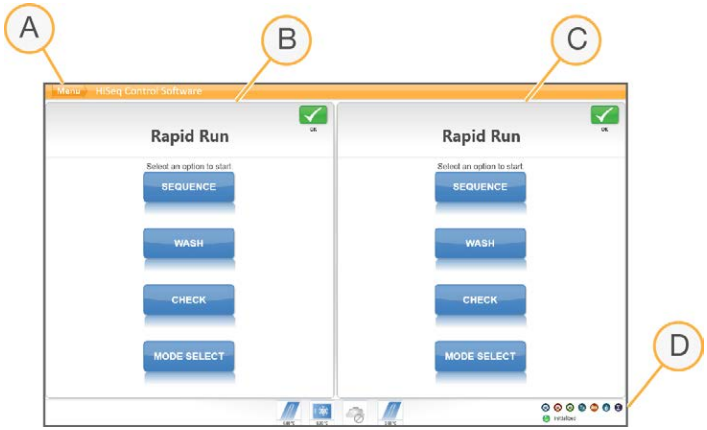
HiSeq 2500 Control Software Interface

The Mode Select screen provides run mode options. Modes include TruSeq v3, HiSeq v4, and Rapid Run. Select a run mode to proceed to the Welcome screen. Because only runs of the same mode can be performed simultaneously, the selected mode is applied to flow cell A and flow cell B.

The Welcome screen is split into 2 panels, 1 for each flow cell. You can set up a run for flow cell A and flow cell B in parallel using the software interface. Runs can also be set up independently using the software interface.

The Welcome screen provides commands to begin a sequencing run, wash the instrument, perform a system check, and change modes. The current mode appears at the top of the screen. When a run is complete, the software prompts to wash the instrument. After the wash, the software returns to the Welcome screen.

Figure 4 Welcome Screen



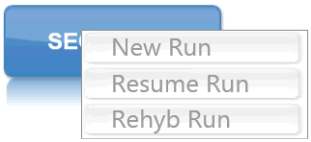
A Welcome screen menu button
B Interface panel for flow cell A
C Interface panel for flow cell B
D Activity indicators

Welcome Screen Commands

The Welcome screen commands include Sequence, Wash, Check, and Mode Select.

- **Sequence**—Select **Sequence** to begin the steps to set up a new sequencing run or resume an existing run.

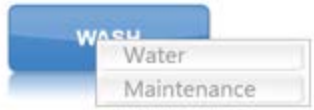
Figure 5 Sequence Command Options



- **New Run**—The software guides you through the steps to specify run parameters, load and prime reagents, load the flow cell, perform fluidics checks, and start the run.
- **Resume Run**—The software guides you through the steps to select the existing run folder and set parameters for resuming the run.
- **Rehyb Run**—The software guides you through the steps to perform on-instrument primer rehybridization. This feature is available in HiSeq v4 and Rapid Run only.

- **Wash**—Select **Wash** to initiate an instrument water wash or maintenance wash.

  Figure 6 Wash Command Options

  - **Water Wash**—The water wash flushes water through the system. This wash is required after a sequencing run or after the instrument has been idle for 1 day or more. See *Perform a Water Wash* on page 104.
  - **Maintenance Wash**—The maintenance wash flushes Tween 20 and ProClin 300 through the system. This wash is required before switching modes or every 10 days, and is a recommended option after a high output run. See *Perform a Maintenance Wash* on page 100.
- **Check**—Select **Check** to open the fluidics check screen and confirm proper flow during instrument installation or fluidics troubleshooting.
- **Mode Select**—Select **Mode Select** to change run modes. Run modes include TruSeq v3, HiSeq v4, and Rapid Run.

Activity and Sensor Indicators

The Welcome screen contains a series of icons in the lower-right corner of the screen that indicate instrument activity and status of specific components based on instrument sensors.

Figure 7 Activity Indicators

From left to right, activity indicators represent the X, Y, and Z motors, electronics functionality, the camera, the fluidics system, and processing functions.

Figure 8 Sensor Indicators

From left to right, sensor indicators represent flow cell A temperature, reagent chiller temperature, BaseSpace cloud status, and flow cell B temperature.

Status Icons

A status icon located in the upper-right corner of each screen shows changes in conditions, errors, or warnings during run setup steps and during the run.

Status Icon	Status Name	Description
	Status OK	No change. System is normal.
	Information	Information only. No action is required
	Attention	Information that might require attention.
	Warning	Warnings do not stop a run, but might require action before proceeding.
	Error	Errors usually stop a run and generally require action before proceeding with the run.

When a change in condition occurs, the associated icon blinks to alert you. To resolve the alert, select the icon to open the status dialog box, which contains a general description of the condition. Select **Acknowledge** to accept the message and **Close** to close the dialog box.

Welcome Screen Menu

The Welcome screen menu button, located in the upper-left corner of the Welcome screen, provides the following options:

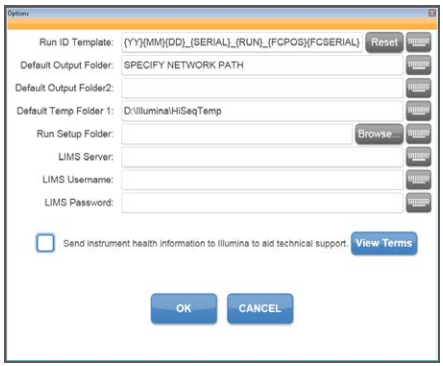
- **View**—Provides options to view the interface in full screen or in a window, or to minimize the interface.
- **Tools**—Provides access to the Options window and Show Log file:
  - **Options**—From the Options window, define the default run settings. See *Menu Options Window* on page 11.
  - **Show Log File**—Lists any errors that occur in the control software. The file is empty unless an error exists. Use this log file for troubleshooting purposes.
- **Scanner**—Activates the command to initialize the software manually.
- **About**—Provides information about instrument hardware, software versions, and technical support contact information.

- **Exit**—Closes the control software interface.

Menu Options Window

The Menu Options window provides settings to define the run ID template, default folder locations, a LIMS server, user name, and password, and whether to send instrument health information to Illumina.

Figure 9 Menu Options Window



- **Run ID Template**—The naming convention used to generate run folder names.
- **Default Output Folder**—The default output folder for runs on flow cell A. This location can be changed on a per run basis.
- **Default Output Folder2**—The default output folder for runs on flow cell B. This location can be changed on a per run basis.
- **Default Temp Folder1**—The location to which temporary files are written during a run.
- **Run Setup Folder**—The location of LIMS sample forms.
- **LIMS Server**—The server name for interactions with supported Illumina LIMS.
- **LIMS User Name**—The user name used when authenticating to Illumina LIMS.
- **LIMS Password**—The password used when authenticating to Illumina LIMS.
- **Send instrument health information to Illumina to aid technical support**—Permits the instrument to send information to BaseSpace for each run. All information remains confidential. Illumina recommends enabling this feature.

Available Disk Space

The HiSeq instrument computer has a storage capacity of over 2.7 TB per flow cell. Data from flow cell A is stored on the D: drive, and data from flow cell B is stored on the E: drive.

At the end of each imaging cycle for each lane, the software checks available disk space on the local D: and E: drives. The software does not check the network location during the run. If disk space drops below the safe threshold, the software pauses the run and places the flow cell in a safe state.

If disk space becomes low, make disk space available to continue the run. When sufficient disk space becomes available, the run resumes automatically.

Sample Sheet Overview

The sample sheet is a user-generated file in *.csv format that stores information about the sequencing run. When the run begins, the software copies the sample sheet to the run folder where it is later used for analysis.

Sample sheets are optional unless you are using BaseSpace to perform data analysis, performing an indexing run, or planning to monitor demultiplexing performance using Sequencing Analysis Viewer. Use the Illumina Experiment Manager (IEM) to create a sample sheet ***before*** you start the run.

Sequencing Consumables

Sequencing on the HiSeq 2500 requires reagents and other consumables provided in Illumina kits. Required kits depend on the type of run to be performed.

- For HiSeq v4 high output runs, see *HiSeq v4 Sequencing Consumables* on page 18.
- For TruSeq v3 high output runs, see *TruSeq v3 Sequencing Consumables* on page 42.
- For Rapid Run, see *Rapid Run Sequencing Consumables* on page 70.

User-Supplied Consumables

Consumable	Supplier	Purpose
Tween 20, viscous liquid, 100 ml	Sigma-Aldrich, catalog # P7949	Instrument maintenance wash.
ProClin 300, 50 ml	Sigma-Aldrich, catalog # 48912-U	Instrument maintenance wash.
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 95041-714 General lab supplier	Cleaning the flow cell and flow cell stage.
Centrifuge tubes, 250 ml	Corning, catalog # 430776	Instrument maintenance wash and water wash.
Conical tubes, 15 ml	Corning, catalog # 430052	Collecting and measuring waste volumes. Instrument maintenance wash and water wash.
Conical tubes, 50 ml, self-standing (optional)	Corning, catalog # 430921	Storing flow cells.
Carboy, at least 6 liters	General lab supplier	Maintenance wash solution.
Disposable gloves, powder-free	General lab supplier	General use.
Lab tissue, low-lint	VWR, catalog # 21905-026	Cleaning the flow cell holder.
Lens paper, 4 x 6 in	VWR, catalog # 52846-001	Cleaning the flow cell.
Pipette tips, 200 µl	General lab supplier	Splitting reagent kit volumes.
Pipette tips, 1000 µl	General lab supplier	Splitting reagent kit volumes.

Consumable	Supplier	Purpose
Tweezers, square plastic tip	McMaster-Carr, catalog # 7003A22	Removing the flow cell gaskets.
Water, laboratory-grade, 18 M Ohm	Millipore	SBS reagent rack, position 2. Instrument wash.

Microcentrifuge Tubes for Rapid Run Mode

Consumable	Supplier
Microcentrifuge tube, 1.5 ml	VWR, catalog # 20170-038, catalog # 20170-650, or catalog # 89000-028 Axygen, catalog # MCT-150-C
Microcentrifuge tube, 1.7 ml	VWR, catalog # 20170-575 Axygen, catalog # MCT-175-C Sorenson BioScience, catalog # 16070

Additional Resources

The following documentation is available for download from the Illumina website.

Resource	Description
HiSeq 2500, 1500, and 2000 Site Prep Guide (part # 15006407)	Provides specifications for laboratory space, electrical requirements, and environmental considerations.
HiSeq Safety and Compliance Guide (part # 15012614)	Provides information about instrument labeling, compliance certifications, and safety considerations.
HiSeq Cluster Kit v4 Reagent Prep Guide (part # 15050104)	Provides a description of cluster kit contents and instructions for preparing consumables before a sequencing run.
HiSeq Rapid Cluster Kit v2 Reagent Prep Guide (part # 15059131)	Provides a description of rapid cluster kit contents and instructions for preparing consumables before a rapid sequencing run.
HiSeq SBS Kit v4 Reagent Prep Guide (part # 15050108)	Provides a description of SBS kit contents and instructions for preparing consumables before a sequencing run.
HiSeq Rapid SBS Kit v2 Reagent Prep Guide (part # 15058772)	Provides a description of rapid SBS kit contents and instructions for preparing consumables before a rapid sequencing run.
Denaturing and Diluting Libraries for the HiSeq and GAIIx (part # 15050107)	Provides instructions for denaturing and diluting prepared libraries for a sequencing run, and preparing a PhiX control. This step applies to most library types.

Visit the HiSeq 2500 support page on the Illumina website for access to documentation, software downloads, online training, and frequently asked questions.

Perform a HiSeq v4 Run

Chapter 2

Introduction

To perform a HiSeq v4 run on the HiSeq 2500, prepare all reagents for the run and then follow the software prompts to set up the run. Run setup steps include entering run parameters, loading and priming reagents, loading the flow cell, and performing a fluidics check.

Visit the HiSeq 2500 specifications page on the Illumina website for information about run duration and other performance specifications.

HiSeq v4 Sequencing Consumables

HiSeq v4 Kit Name	Description
HiSeq SBS Kit v4	Contains SBS reagents used on the HiSeq 2500.
HiSeq PE Cluster Kit v4 or HiSeq SR Cluster Kit v4	Contains clustering reagents used on the cBot and indexing reagents used on the HiSeq 2500. The PE version of the cluster kit includes paired-end reagents used on the HiSeq 2500. Each cluster kit includes an accessories kit that contains flow cell gasket replacements and funnel caps for SBS reagent bottles.

Reagent Preparation Steps

Before setting up the run, prepare SBS reagents, indexing reagents, and paired-end reagents, if applicable.

- For SBS reagent preparation, see the *HiSeq SBS Kit v4 Reference Guide (part # 15050108)*.
- For indexing and paired-end reagent preparation, see the *HiSeq Cluster Kit v4 Reference Guide (part # 15050104)*.

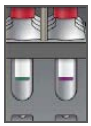
Prepare all reagents before setting up the run. When prompted by the control software, load all reagents. When using HiSeq v4 chemistry, there is no need to return to the instrument during the run to load reagents.

HiSeq v4 Sequencing Workflow

Prepare reagents for the run. Weigh reagents after preparation.

For reagent preparation information, see *Reagent Preparation Steps* on page 18.

Using the control software, enter run parameters.



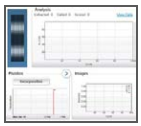
When prompted, load all reagents for the run:

- Load SBS reagents for Read 1 and Read 2.
- For indexed runs, load indexing reagents.
- For paired-end runs, load paired-end reagents.

With a used flow cell on the instrument, confirm proper flow.

Prime SBS reagents and measure priming waste.

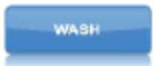
Load the clustered flow cell for sequencing. Confirm proper flow.



Start the sequencing run.

[Optional] After cycle 1, inspect the first base report, and then continue Read 1.

The run continues as specified in run parameters.



When the run is complete, unload and weigh reagents.

Perform an instrument wash.

Run Types for HiSeq v4 Chemistry

The following table shows types of sequencing runs and the number of possible cycles for each read when using HiSeq v4 chemistry. Use this information as a reference when setting up the run.

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 126	--	--	--	≤ 126
Single-Read, Single-Indexed	≤ 126	6 or 7 [1] 8 [2]	--	--	≤ 133 [1] ≤ 134 [2]
Single-Read, Dual-Indexed	≤ 126	8	8	--	≤ 142
Paired-End, Non-Indexed	≤ 126	--	--	≤ 126	≤ 252
Paired-End, Single-Indexed	≤ 126	7 [1] 8 [2]	--	≤ 126	≤ 259 [1] ≤ 260 [2]
Paired-End, Dual-Indexed	≤ 126	8	7 + 8 [3]	≤ 126	≤ 275

[1] Number of cycles for single-indexed libraries
[2] Number of cycles for dual-indexed libraries
[3] Index 2 Read of a paired-end dual-indexed run includes 7 additional chemistry-only cycles

Enter Run Parameters

From the Welcome screen, select **Sequence** | **New Run**.

The control software interface guides you through the steps to set up the run. Run setup steps are organized in 3 tabs: Run Configuration, Pre-Run Setup, and Initiate Run.

- Run configuration screens contain drop-down lists, checkboxes, or text fields for run parameters. Use the hand-held barcode scanner to scan the flow cell or reagent kit ID, or enter the ID using the touch screen keyboard. The keyboard icon is located to the right of the text fields. 
- Select **Next** to move to the next screen, or select **Back** to return to the previous screen.
- At any time during the run setup steps, select **Cancel** to exit run setup and return to the Welcome screen.

Integration Screen

The Integration screen provides the option to connect the run to BaseSpace.

To connect to BaseSpace, do the following:

1. Select **BaseSpace**.
2. Select from the following BaseSpace options:
   - **Storage and Analysis**—Sends run data to BaseSpace for remote monitoring and data analysis. A sample sheet is required with this option.
   - **Run Monitoring Only**—Sends only InterOp files to BaseSpace, which allows remote monitoring of the run.
3. Log on to BaseSpace using your MyIllumina account email and password.
4. Select **Next**.

To proceed without connecting to BaseSpace, do the following:

1. Select **None**.
2. Select **Next**.

Storage Screen

1. Select the **Save to an output folder** checkbox, and select **Browse** to navigate to a preferred network location. If the run is connected to BaseSpace for storage and

analysis, this field is optional.

2 Select **Zip BCL files** to reduce required storage space. If the run is connected to BaseSpace, the **Zip BCL files** option is selected by default.

NOTE
The **Bin Q-Scores** setting is enabled by default to reduce required storage space. This setting groups quality scores over a wider range of values without affecting accuracy or performance.

3 Select from the following Save Auxiliary Files options:
- **Save All Thumbnails**—Saves all thumbnails images. A thumbnail is a sampling of images from many tiles in each column of tiles, or swath, combined in 1 thumbnail image.
- **Save Tile Thumbnails**—Saves tile thumbnails. Tile thumbnails represent a single tile rather than a sampling of tiles in a swath.

4 Select **Next**.

Flow Cell Setup Screen

The Flow Cell Setup screen records information about the flow cell used for the run. All fields are required.

1 Scan the flow cell barcode or enter the flow cell ID (barcode number) of the flow cell to be sequenced. The flow cell ID is used to determine flow cell type and reagent compatibility.

2 Confirm that the flow cell type is **HiSeq Flow Cell v4**. The flow cell type is selected automatically based on the flow cell ID.

3 Enter an experiment name. The experiment name appears on each screen to help identify the run in progress.

4 Enter a user name.

5 Select **Next**.

Advanced Screen

1 [Optional] Select the **Confirm First Base** checkbox.

A first base report is generated automatically for each run. Selecting this option opens the first base report before proceeding with the run.

2 [Optional] From the **Align to PhiX** checkboxes, clear the checkbox for lanes that do not contain PhiX.
By default, all lanes are selected for alignment by Real-Time Analysis software. Alternatively, select lanes on the flow cell image to add or remove lanes for PhiX alignment.

NOTE
A dedicated control lane is not required with HCS v2.2 and RTA v1.18. Therefore, the option to assign a control lane is not available with this software configuration.

3 Select **Next**.

Recipe Screen

1 Select from the following Index Type options:
- **No Index**—Performs a non-indexed single-read or paired-end run.
- **Single Index**—Performs a single-read or paired-end run with 1 indexing read.
- **Dual Index**—Performs a single-read or paired-end run with 2 indexing reads.
- **Custom**—Performs a single-read or paired-end run with a custom number of cycles for index reads.

2 If the Dual Index or Custom option is specified, select a Flow Cell Format, either **Single Read** or **Paired End**.

3 Enter the number of cycles for Read 1 and Read 2, if applicable.

NOTE
The number of cycles performed in a read is 1 more cycle than the number of cycles analyzed. For example, to perform 125 cycles for Read 1, enter 126.

For the **Custom** indexing option, enter the number of cycles for index reads. Read lengths do not need to be identical.

4 Confirm the following default chemistry settings. These fields are auto-populated depending on the selected index type option.
a **SBS**: **HiSeq SBS Kit v4**
b **Index**: **HiSeq v4 Single Index** or **HiSeq v4 Dual Index**
c **PE turnaround**: **HiSeq PE Cluster Kit v4**

5 [Optional] Select the **Use Existing Recipe** checkbox to use a custom recipe. Otherwise,

allow the software to create the recipe from the run parameters entered.

Sample Sheet Screen

Sample sheets are optional unless you use BaseSpace to perform data analysis or perform an indexed run.

1 Select **Browse** to navigate to the sample sheet location.

2 Select **Next**.

NOTE
HiSeq Control Software v2.2 allows a different indexing scheme in each lane.

Reagents Screen

The Reagents screen records information about reagent kits used for the run. The reagent kit ID (barcode number beginning with **RGT**) is used to determine reagent kit type and run mode compatibility.

1 Scan or enter the SBS reagent kit ID.

2 For paired-end runs, scan or enter the reagent kit ID for paired-end cluster kit.

3 Select the SBS reagent kit for the run:

- Select **250 Cycles** for a 250 cycle kit. Cycles remaining defaults to 275.
- Select **50 Cycles** for a 50 cycle kit. Cycles remaining defaults to 74.
- Select **Custom** for a partial kit or multiple 50-cycle kits. In the Cycles Remaining field, enter the number of SBS cycles that reagents are expected to last.

NOTE
For partial kits, the software counts down the number of cycles entered. When the cycles are low, the software prompts you to load fresh reagents.

4 Select **Prime SBS Reagents** to prime reagents before starting a run. Always prime reagents before loading a new flow cell.

5 Select **Next**.

Review Screen

1 Review the run parameters on the Review screen.

2 Select **Next** to proceed or select **Back** to change parameters.

Load and Prime Reagents

After entering run parameters, load SBS, indexing, and paired-end reagents for the run, and then prime reagents through the fluidics system. The software guides you through these steps in a series of screens on the Pre-Run Setup tab.

Illumina-Supplied Consumables

- 8 funnel caps

User-Supplied Consumables

- 250 ml bottle (Corning, catalog # 430776)
- 15 ml conical tubes (Corning, catalog # 430052)
- Laboratory-grade water

NOTE
To prepare for the post-run rinse at the end of a sequencing run, load 25 ml PW1 or laboratory-grade water in position 2.
The post-run rinse does ***not*** replace the post-run instrument wash.

Load SBS Reagents

1 Invert each bottle several times to make sure that the reagents are mixed thoroughly.

2 Remove the cap from each reagent bottle and replace it with a funnel cap.

CAUTION
After handling the bottle of CRM, discard your gloves and replace them with a new pair.

3 Record the weight of each reagent on the lab tracking form.

NOTE
Weighing reagents before and after a sequencing run confirms proper reagent delivery.

4 Open the reagent compartment door.

5 Raise the sippers for the sequencing reagent rack using the following motion:
   a Pull the handle towards you and then raise the handle.
   b Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.

6 Slide the reagent rack out of the reagent compartment.

7 Place each reagent bottle onto the rack in the associated numbered position. Make sure that the conical end of the bottle rests in the indentation on the base of the rack.

Table 1 Reagent Positions

Position	Reagent	Description
1	IRM	Incorporation Reagent Master Mix
2	PW1	25 ml of PW1 or laboratory-grade water
3	USM	Universal Scan Mix
4	SBS Buffer 1 (SB1)	High Salt Buffer
5	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
6	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
7	CRM	Cleavage Reagent Mix
8	SBS Buffer 3 (SB3)	Cleavage Buffer

8 Add 25 ml of PW1 or laboratory-grade water to the bottle in position 2.

9 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

10 Lower the sippers into the sequencing reagent bottles as follows:
   a Pull the sipper handle towards you and then lower the sipper handle.
   b Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
   c Release the sipper handle into the slot on the bottom end of the groove.

11 Select the **PW1 (25 ml) loaded** checkbox.

Load Indexing Reagents

1 Record the weight of each reagent on the lab tracking form.

2 Make sure that the paired-end rack is not in use on the adjacent flow cell. Steps that use the paired-end rack include Read 2 resynthesis, Index 1 (i7) Read preparation, and Index 2 (i5) Read preparation.

3 Raise the sippers for the paired-end reagent rack using the following motion:
   a Pull the handle towards you and then raise the handle.
   b Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.

4 Slide the reagent rack out of the reagent compartment using the rack handle.

5 Remove the caps from each reagent tube and place the tube onto the rack in the associated numbered position or matching label color.

Table 2 Single-Read Flow Cells

Position	Reagent	Description
15	FDR	Fast Denaturation Reagent (contains formamide)
16	HP9 *	Index Sequencing Primer i5
17	HP12	Index Sequencing Primer i7

* HP9 is required for dual-indexed runs only. If HP9 is not used, load a 15 ml conical tube with 10 ml laboratory-grade water in position 16.

Table 3 Paired-End Flow Cells

Position	Reagent	Description
10	FRM *	Fast Resynthesis Mix
15	FDR	Fast Denaturation Reagent (contains formamide)
17	HP12	Index Sequencing Primer i7

* Load FRM in position 10 for dual-indexed runs on a paired-end flow cell. FRM is required in position 10 for all paired-end runs regardless of indexing options.

6 If you are performing a single-read run, proceed with the following steps to return the rack to the reagent compartment. Otherwise, proceed to loading paired-end reagents.

7 Place 15 ml conical tubes filled with 10 ml laboratory-grade water in unused positions on the paired-end rack.

8 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

9 If you are performing a single-read run, lower the sippers into the paired-end reagent tubes as follows:

a Pull the handle towards you and then lower the handle.

b Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.

c Release the handle into the slot on the bottom end of the groove.

10 Select **Next**.

Load Paired-End Reagents

1. Record the weight of each reagent on the lab tracking form.
2. Raise the sippers for the paired-end reagent rack using the following motion:
   a. Pull the handle towards you and then raise the handle.
   b. Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.
3. Slide the reagent rack out of the reagent compartment using the rack handle.
4. Remove the caps from each reagent tube and place the tube onto the rack in the associated numbered position or matching label color.

Table 4 Paired-End Flow Cells

Position	Reagent	Description
10	FRM *	Fast Resynthesis Mix
11	FLM2	Fast Linearization Mix 2
13	AMS	Fast Amplification Mix
14	FPM	Fast Amplification Premix
15	FDR *	Fast Denaturation Reagent (contains formamide)
16	HP11	Read 2 Sequencing Primer

* If you loaded indexing reagents for a single-index run, FDR is already loaded in position 10. If you loaded indexing reagents for a dual-index run, FRM and FDR are already loaded in positions 10 and 15, respectively.

5. Place 15 ml conical tubes filled with 10 ml laboratory-grade water in unused positions on the paired-end rack.
6. Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.
7. Lower the sippers into the paired-end reagent tubes as follows:
   a. Pull the handle towards you and then lower the handle.
   b. Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.
   c. Release the handle into the slot on the bottom end of the groove.
8. Select **Next**.

Prime Reagents

Steps for priming reagents include cleaning the flow cell holder, loading a priming flow cell, confirming proper flow, and then starting the prime.

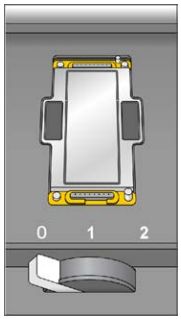
Clean the Flow Cell Holder

1 Open the flow cell compartment door.

CAUTION
Do not place fluids on the flow cell compartment door or on the flow cell stage when the door is open. Spills in this area can damage the instrument.

2 Make sure that the flow cell lever is in the OFF position.

Figure 10 Flow Cell Lever in Position 0



3 Put on a new pair of powder-free latex gloves.

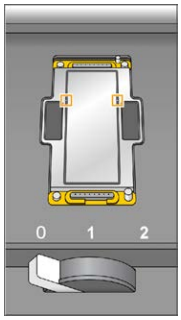
4 If the flow cell from a previous run is present, remove it and set aside in a tube of storage buffer or laboratory-grade water to keep it from drying out. It can be used to confirm proper flow before loading the clustered flow cell.

5 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

6 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Figure 11 Vacuum Hole Locations



Load a Priming Flow Cell

From the Load Priming Flow Cell screen, load a ***used*** flow cell for the priming step. After loading a used flow cell, confirm that the vacuum is engaged.

NOTE
Illumina recommends using the flow cell from a previous run for priming reagents on a subsequent run or for a post-run instrument wash.

1 Rinse the used flow cell with laboratory-grade water. Dry the flow cell with a lens cleaning tissue or lint-free tissue.

2 Clean the flow cell using alcohol wipes and lens cleaning tissue.

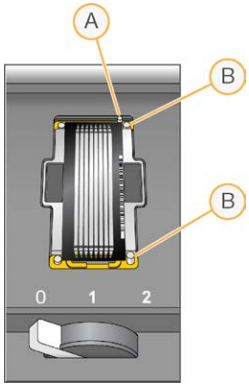
NOTE
Do not remove or replace the flow cell gaskets during this step.

3 Place the used flow cell on the flow cell holder with the inlet and outlet ports facing ***down*** and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

4 Gently slide the flow cell towards the top and right guide pins until it stops.

NOTE
Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.

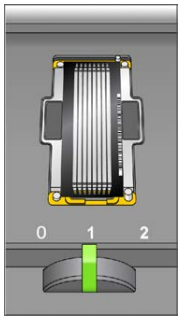
Figure 12 Flow Cell Positioned Against Top and Right Guide Pins



A Top guide pin
B Right guide pins

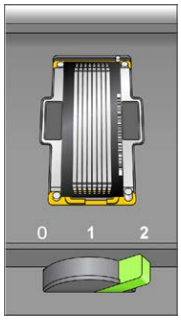
5 Slowly move the flow cell lever to position 1, which engages the vacuum and secures the flow cell into position. When the flow cell lever is blinking green, the vacuum is engaged. If the lever is not green, see *Possible Run Setup Problems* on page 129.

Figure 13 Flow Cell Lever in Position 1



6 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2 (far-right). When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 14 Flow Cell Lever in Position 2



7 Make sure that the **Vacuum Engaged** checkbox is selected on the load prime flow cell screen, and then select **Next**.

Confirm Proper Flow

Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 2 (laboratory-grade water) from the drop-down list.

CAUTION
Use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

2 Confirm the following default values:
- Volume: **125**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

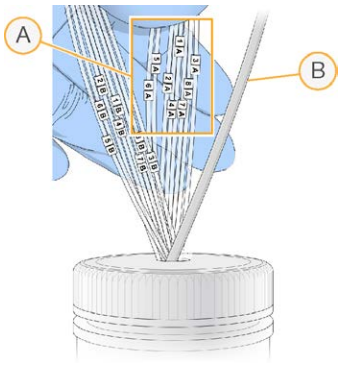
3 Select **Pump**.

4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
If excessive bubbles are present, check the gaskets for obstructions, reduce the aspirate rate to 100, and pump another 125 µl of water to the flow cell. If problems persist, remove the flow cell, repeat the cleaning steps, and reload the flow cell.

Position Tubing and Start Prime

1 Remove the 8 waste tubes for the appropriate flow cell from the waste container. Do not include the 8 tubes for the opposite flow cell or the tube for the condensation pump.

Figure 15 Position Tubing



A Flow cell waste tubes for reagent positions 1–8
B Condensation pump tubing (do not remove)

2 Place waste tubing into an empty 15 ml tube, 1 waste tube per 15 ml tube. Priming waste is collected and measured after the priming step.

3 Select **Start Prime**. The priming screen opens and the priming step begins. Monitor the progress of the priming step from the priming screen.

4 When the priming step is complete, measure the collected waste and confirm that the volume in each tube is 1.75 ml for a total of **14 ml**. The total is calculated as follows:
- 250 µl for each SBS position except position 2 (250 x 7 = 1.75 ml)
- 1.75 ml for each lane (1.75 x 8 = 14 ml)

5 Record the results on the lab tracking form.

6 Return the waste tubing to the waste container before proceeding.

7 Select **Next**.

Load a Flow Cell

Steps to load the clustered flow cell include removing the priming flow cell, cleaning the flow cell holder, cleaning the flow cell, loading the flow cell, and confirming proper flow.

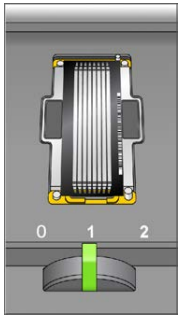
User-Supplied Consumables

- Lens cleaning tissue
- 70% ethanol or alcohol wipes
- Low-lint lab tissue
- One pair of plastistats

Remove the Used Flow Cell

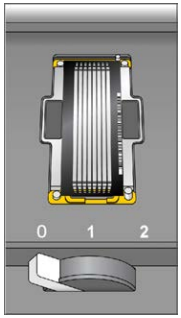
1 Slowly move the flow cell lever to position 1 to disengage the manifolds.

Figure 16 Flow Cell Lever in Position 1



2 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

Figure 17 Flow Cell Lever in Position 0



3 Lift the used flow cell from the flow cell holder.

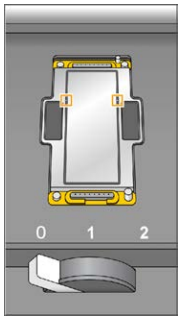
Clean the Flow Cell Holder

1 Put on a new pair of powder-free latex gloves.

2 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

Figure 18 Inspect Vacuum Holes



3 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Clean the Flow Cell

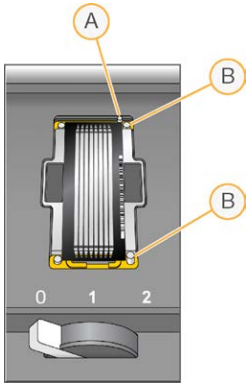
1. Remove the flow cell from the flow cell container using a pair of plastistats.
2. Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.
3. Fold an alcohol wipe to approximately the size of the flow cell.
4. Hold the edges of the clustered flow cell with 2 fingers. Make sure that the inlet and outlet ports are facing ***up***.
5. Wipe each side of the flow cell with a single sweeping motion. Repeat, refolding the alcohol wipe with each pass, until the flow cell is clean.
6. Dry the flow cell using a dry lens cleaning tissue.
7. Protect the flow cell from dust until you are ready to load it onto the instrument.

Load the Sequencing Flow Cell

NOTE
Do not replace the manifold gaskets. Replace the manifold gaskets after the sequencing run is complete and before the maintenance wash.

1. Place the flow cell on the flow cell holder with the inlet and outlet ports facing ***down*** and the barcode on the right. Make sure that the arrow on the left edge of the flow cell points towards the instrument.
2. Gently slide the flow cell towards the top and right guide pins until it stops.

Figure 19 Flow Cell Positioned Against Top and Right Guide Pins

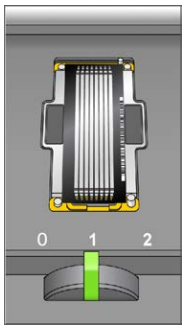


A Top guide pin
B Right guide pins

NOTE
Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.

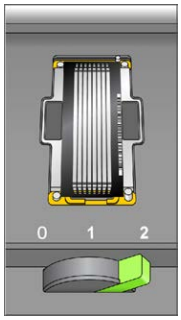
3 Slowly move the flow cell lever to position 1, which engages the vacuum and secures the flow cell into position. When the flow cell lever is blinking green, the vacuum is engaged. If the lever is not green, see *Possible Run Setup Problems* on page 129.

Figure 20 Flow Cell Lever in Position 1



4 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 21 Flow Cell Lever in Position 2



5 Make sure that the **Vacuum Engaged** checkbox is selected on the Load Sequencing Flow Cell screen.

Confirm Proper Flow

Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 5 from the drop-down list.

2 Enter the following default values:
- Volume: **250**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

3 Select **Pump**.

4 Visually inspect the flow cell for bubbles passing through the lanes or leaks near the manifolds.
If excessive bubbles are present, check the manifold gaskets for obstructions and repeat the process using solution 6 to avoid depleting position 5. Reduce the aspirate rate to 100, and pump another 250 µl to the flow cell.

5 Select **Next**. Make sure that the flow cell lever is green, and then close the flow cell compartment door.

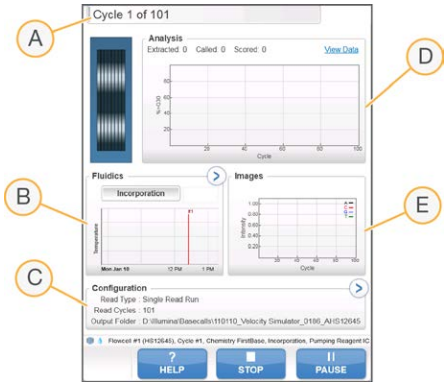
6 Confirm that the checkboxes **Vacuum Engaged** and **Door Closed** are selected, and then select **Next**.

7 Select **Start** to start the sequencing run.

Monitor the Run

Monitor run metrics on the run overview screen, fluidics, and imaging.

Figure 22 Run Overview Screen



A **Progress bar**—Use the progress bar to monitor how many cycles have been completed.
B **Fluidics graph**—Expand the fluidics section to monitor chemistry steps.
C **Run Configuration**—Review parameters of current run.
D **Analysis graph**—Use the analysis graph to monitor quality scores by cycle.
E **Images graph**—Use the images graph to monitor intensities by cycle.

First Base Report

If you selected the **Confirm First Base** option during run setup, the first base confirmation dialog box opens automatically after imaging of the first cycle is complete. The run pauses at this step.

1 Review the First Base Report from the confirmation dialog box.

2 If the results are satisfactory, select **Continue**.

Post-Run Procedures

When the run is complete, unload and weigh reagents, and then perform an instrument wash. For more information, see *Post-Run Procedures* on page 97.

Perform a TruSeq v3 Run

Chapter 3

Introduction

To perform a TruSeq v3 run on the HiSeq 2500, prepare SBS reagents for Read 1 and indexing reagents before setting up the run. Follow the software prompts to set up the run, which includes entering run parameters, loading and priming reagents, loading the flow cell, and performing a fluidics check.

Prepare and load paired-end reagents and SBS reagents for Read 2 after the completion of Read 1 and any index reads.

Visit the HiSeq 2500 specifications page on the Illumina website for information about run duration and other performance specifications.

TruSeq v3 Sequencing Consumables

TruSeq v3 Kit Name	Description
TruSeq SBS Kit v3 (200 Cycles) or TruSeq SBS Kit v3 (50 Cycles)	Contains SBS reagents used on the HiSeq 2500.
TruSeq PE Cluster Kit v3 or TruSeq SR Cluster Kit v3	Contains clustering reagents used on the cBot and indexing reagents used on the HiSeq 2500. The PE version of the cluster kit includes paired-end reagents used on the HiSeq 2500. Each cluster kit includes an accessories kit that contains flow cell gasket replacements and funnel caps for SBS reagent bottles.

Reagent Preparation Steps

- For SBS reagent preparation, see the appropriate guide:
  - *TruSeq SBS Kit v3 Reagent Prep Guide (200 Cycles) (part # 15023333)*
  - *TruSeq SBS Kit v3 Reagent Prep Guide (50 Cycles) (part # 15023334)*
- For indexing and paired-end reagent preparation, see the appropriate guide:
  - *TruSeq PE Cluster Kit v3 Reagent Prep Guide (part # 15023336)*
  - *TruSeq SR Cluster Kit v3 Reagent Prep Guide (part # 15023335)*

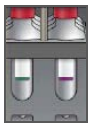
  These guides include instructions for preparing sequencing primers provided in the TruSeq Dual Index Sequencing Primer Box.

TruSeq v3 Sequencing Workflow

Prepare SBS reagents for Read 1 and indexing reagents. Weigh reagents after preparation.

For reagent preparation information, see *Reagent Preparation Steps* on page 42.

Using the control software, enter run parameters.



When prompted, load all SBS reagents for Read 1.

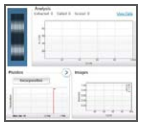
Load SBS reagents for Read 2, except ICB.

Load indexing reagents.

With a used flow cell on the instrument, confirm proper flow.

Prime SBS reagents and measure priming waste.

Load the clustered flow cell for sequencing. Confirm proper flow.



Start the sequencing run.

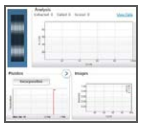
[Optional] After cycle 1, inspect the first base report, and then continue Read 1.

The run continues as specified in run parameters.

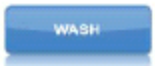
Prepare paired-end reagents and fresh ICB for Read 2. Weigh reagents after preparation.

For reagent preparation information, see *Reagent Preparation Steps* on page 42.

Load paired-end reagents and fresh ICB for Read 2.



Continue the run. The software automatically primes paired-end reagents and performs Read 2 resynthesis and Read 2.



When the run is complete, unload and weigh reagents.

Perform an instrument wash.

Run Types for TruSeq v3 Chemistry

The following table shows types of sequencing runs and the number of possible cycles for each read when using TruSeq v3 chemistry. Use this information as a reference when setting up the run.

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 101	--	--	--	≤ 101
Single-Read, Single-Indexed	≤ 101	6 or 7 [1] 8 [2]	--	--	≤ 108 [1] ≤ 109 [2]
Single-Read, Dual-Indexed	≤ 101	8	8	--	≤ 117
Paired-End, Non-Indexed	≤ 101	--	--	≤ 101	≤ 202
Paired-End, Single-Indexed	≤ 101	7 [1] 8 [2]	--	≤ 101	≤ 209 [1] ≤ 210 [2]
Paired-End, Dual-Indexed	≤ 101	8	7 + 8 [3]	≤ 101	≤ 225

[1] Number of cycles for single-indexed libraries
[2] Number of cycles for dual-indexed libraries
[3] Index 2 Read of a paired-end dual-indexed run includes 7 additional chemistry-only cycles

Enter Run Parameters

From the Welcome screen, select **Sequence** | **New Run**.

The control software interface guides you through the steps to set up the run. Run setup steps are organized in 3 tabs: Run Configuration, Pre-Run Setup, and Initiate Run.

- Run configuration screens contain drop-down lists, checkboxes, or text fields for run parameters. Use the hand-held barcode scanner to scan the flow cell or reagent kit ID, or enter the ID using the touch screen keyboard. The keyboard icon is located to the right of the text fields. 
- Select **Next** to move to the next screen, or select **Back** to return to the previous screen.
- At any time during the run setup steps, select **Cancel** to exit run setup and return to the Welcome screen.

Integration Screen

The Integration screen provides the option to connect the run to BaseSpace.

To connect to BaseSpace, do the following:

1. Select **BaseSpace**.
2. Select from the following BaseSpace options:
   - **Storage and Analysis**—Sends run data to BaseSpace for remote monitoring and data analysis. A sample sheet is required with this option.
   - **Run Monitoring Only**—Sends only InterOp files to BaseSpace, which allows remote monitoring of the run.
3. Log on to BaseSpace using your MyIllumina account email and password.
4. Select **Next**.

To proceed without connecting to BaseSpace, do the following:

1. Select **None**.
2. Select **Next**.

Storage Screen

1. Select the **Save to an output folder** checkbox, and select **Browse** to navigate to a preferred network location. If the run is connected to BaseSpace for storage and

analysis, this field is optional.

2 Select **Zip BCL files** to reduce required storage space. If the run is connected to BaseSpace, the **Zip BCL files** option is selected by default.

NOTE
The **Bin Q-Scores** setting is enabled by default to reduce required storage space. This setting groups quality scores over a wider range of values without affecting accuracy or performance.

3 Select from the following Save Auxiliary Files options:
- **Save All Thumbnails**—Saves all thumbnails images. A thumbnail is a sampling of images from many tiles in each column of tiles, or swath, combined in 1 thumbnail image.
- **Save Tile Thumbnails**—Saves tile thumbnails. Tile thumbnails represent a single tile rather than a sampling of tiles in a swath.

4 Select **Next**.

Flow Cell Setup Screen

The Flow Cell Setup screen records information about the flow cell used for the run.

1 Scan the flow cell barcode or enter the flow cell ID (barcode number) of the flow cell to be sequenced. The flow cell ID is used to determine flow cell type and reagent compatibility.

2 Confirm that the flow cell type is **HiSeq Flow Cell v3**, which is automatically selected based on the flow cell ID.

3 Enter an experiment name. The experiment name appears on each screen to help identify the run in progress.

4 Enter a user name.

5 Select **Next**.

Advanced Screen

1 [Optional] Select the **Confirm First Base** checkbox.
A first base report is generated automatically for each run. Selecting this option opens the first base report before proceeding with the run.

2 [Optional] From the **Align to PhiX** checkboxes, clear the checkbox for lanes that do not contain PhiX.
By default, all lanes are selected for alignment by Real-Time Analysis (RTA). Alternatively, select lanes on the flow cell image to add or remove lanes for PhiX alignment.

NOTE
A dedicated control lane is not required with HCS v2.2 and RTA v1.18. Therefore, the option to assign a control lane is not available with this software configuration.

3 [Optional] Select **Keep Intensity Files** for later reanalysis or custom processing.
By default, this option is not selected. Saving intensity files is not required for on-instrument analysis. Enabling this option significantly increases the size of the data output folder.

4 Select **Next**.

Recipe Screen

A recipe is generated automatically from the information entered on the Recipe screen.

1 Select 1 of the following Index Type options:
- **No Index**—Performs a non-indexed single-read or paired-end run.
- **Single Index**—Performs a single-read or paired-end run with 1 indexing read.
- **Dual Index**—Performs a single-read or paired-end run with 2 indexing reads.
- **Custom**—Performs a single-read or paired-end run with a custom number of cycles for index reads.

2 If the Dual Index or Custom option is specified, select a Flow Cell Format, either **Single Read** or **Paired End**.

3 Enter the number of cycles for Read 1 and Read 2, if applicable.

NOTE
The number of cycles performed in a read is 1 more cycle than the number of cycles analyzed. For example, to perform 125 cycles for Read 1, enter 126.

For the **Custom** indexing option, enter the number of cycles for index reads. Read lengths do not need to be identical.

4 Confirm the following default chemistry settings. These fields are auto-populated depending on the selected index type option.

a **SBS**: **TruSeq SBS Kit v3**
b **Index**: **TruSeq Multiplex Sequencing Primer Box** or **TruSeq Dual Index Sequencing Primer Box**
c **PE turnaround**: **TruSeq PE Cluster Kit v3**

5 [Optional] Select the **Use Existing Recipe** to use a custom recipe. Otherwise, allow the software to create the recipe from run parameters entered.

Sample Sheet Screen

Sample sheets are optional unless you use BaseSpace to perform data analysis or perform an indexed run.

1 Select **Browse** to navigate to the sample sheet location.

2 Select **Next**.

NOTE
HiSeq Control Software v2.2 allows a different indexing scheme in each lane.

Reagents Screen

The Reagents screen records information about reagent kits used for the run. The reagent kit ID (barcode number beginning with **RGT**) is used to determine reagent kit type and run mode compatibility.

1 Scan or enter the SBS reagent kit ID.

2 For paired-end runs, scan or enter the reagent kit ID for paired-end cluster kit.

3 Select the SBS reagent kit for the run:
- Select **200 Cycles** for a 200 cycle kit. Cycles remaining defaults to 209.
- Select **50 Cycles** for a 50 cycle kit. Cycles remaining defaults to 59.
- Select **Custom** for a partial kit or multiple 50-cycle kits. In the Cycles Remaining field, enter the number of SBS cycles that reagents are expected to last.

NOTE
For partial kits, the software counts down the number of cycles entered. When the cycles are low, the software prompts you to load fresh reagents.

4 Select **Prime SBS Reagents** to prime reagents before starting a run. Always prime reagents before loading a new flow cell.

5 Select **Next**.

Review Screen

1. Review the run parameters on the Review screen.
2. Select **Next** to proceed or select **Back** to change parameters.

Load and Prime Reagents

After entering run parameters, load SBS and indexing reagents for the run, and then prime reagents through the fluidics system. The software guides you through these steps in a series of screens on the Pre-Run Setup tab.

Illumina-Supplied Consumables

- Eight funnel caps

User-Supplied Consumables

- 250 ml bottle (Corning, catalog # 430776)
- 15 ml conical tubes (Corning, catalog # 430052)
- Laboratory-grade water

NOTE
To prepare for the post-run rinse at the end of a sequencing run, load 25 ml PW1 or laboratory-grade water in position 2.
The post-run rinse does ***not*** replace the post-run instrument wash.

Load SBS Reagents

1 Remove the cap from each reagent bottle and replace it with a funnel cap.

CAUTION
After handling the bottle of CMR, discard your gloves and replace them with a new pair.

2 Record the weight of each reagent on the lab tracking form.

NOTE
Weighing reagents before and after a sequencing run confirms proper reagent delivery.

3 Open the reagent compartment door.

4 Raise the sippers for the sequencing reagent rack using the following motion:
   a Pull the handle towards you and then raise the handle.
   b Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.

5 Slide the reagent rack out of the reagent compartment.

6 Place each reagent bottle onto the rack in the associated numbered position. Make sure that the conical end of the bottle rests in the indentation on the base of the rack.

Table 5 SBS Reagent Positions

Position	Reagent	Description
1	ICB	Incorporation Mix
2	PW1 (25 ml)	Wash Buffer
3	SRE	Scan Mix Reagent
4	SBS Buffer 1 (SB1)	High Salt Buffer
5	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
6	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
7	CMR	Cleavage Mix Reagent
8	SBS Buffer 3 (SB3)	Cleavage Buffer

7 Add 25 ml of PW1 or laboratory-grade water to the bottle in position 2.

8 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

9 Lower the sippers into the sequencing reagent bottles as follows:
   a Pull the sipper handle towards you and then lower the sipper handle.
   b Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
   c Release the sipper handle into the slot on the bottom end of the groove.

10 Select the **PW1 (25 ml) loaded** checkbox.

Load Indexing Reagents

1 Record the weight of each reagent on the lab tracking form.

2 Make sure that the paired-end rack is not in use on the adjacent flow cell. Steps that use the paired-end rack include Read 2 resynthesis, Index 1 (i7) Read preparation, and Index 2 (i5) Read preparation.

3 Raise the sippers for the paired-end reagent rack using the following motion:
   a Pull the handle towards you and then raise the handle.
   b Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.

4 Slide the reagent rack out of the reagent compartment using the rack handle.

5 Remove the caps from each reagent tube and place the tube onto the rack in the associated numbered position or matching label color.

Table 6 Single-Indexed Run on a Single-Read Flow Cell or Paired-End Flow Cell

Position	Reagent	Description
17	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix
18	HP3	Denaturation Solution
19	HT2	Wash Buffer

Table 7 Dual-Indexed Run on a Single-Read Flow Cell

Position	Reagent	Description
16	HP9	Index 2 (i5) SR Sequencing Primer Mix
17	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix
18	HP3	Denaturation Solution
19	HT2	Wash Buffer

Table 8 Dual-Indexed Run on a Paired-End Flow Cell

Position	Reagent	Description
10	RMR	Resynthesis Mix
17	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix
18	HP3	Denaturation Solution
19	HT2	Wash Buffer

6 Place 15 ml conical tubes filled with 10 ml laboratory-grade water in unused rack positions.

7 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

8 Lower the sippers into the tubes on the paired-end reagent rack as follows:

a Pull the handle towards you and then lower the handle while pulling it towards you.

b Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.

c Release the handle into the slot on the bottom end of the groove.

9 Close the reagent compartment door.

10 Select **Next**.

Prime Reagents

Steps for priming reagents include cleaning the flow cell holder, loading a priming flow cell, confirming proper flow, and then starting the prime.

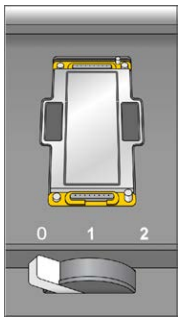
Clean the Flow Cell Holder

1 Open the flow cell compartment door.

CAUTION
Do not place fluids on the flow cell compartment door or on the flow cell stage when the door is open. Spills in this area can damage the instrument.

2 Make sure that the flow cell lever is in the OFF position.

Figure 23 Flow Cell Lever in Position 0



3 Put on a new pair of powder-free latex gloves.

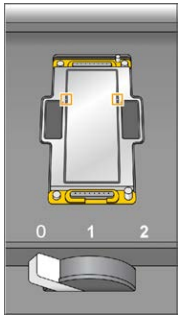
4 If the flow cell from a previous run is present, remove it and set aside in a tube of storage buffer or laboratory-grade water to keep it from drying out. It can be used to confirm proper flow before loading the clustered flow cell.

5 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

6 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Figure 24 Vacuum Hole Locations



Load a Priming Flow Cell

From the Load Priming Flow Cell screen, load a ***used*** flow cell for the priming step. After loading a used flow cell, confirm that the vacuum is engaged.

NOTE
Illumina recommends using the flow cell from a previous run for priming reagents on a subsequent run or for a post-run instrument wash.

1 Rinse the used flow cell with laboratory-grade water. Dry the flow cell with a lens cleaning tissue or lint-free tissue.

2 Clean the flow cell using alcohol wipes and lens cleaning tissue.

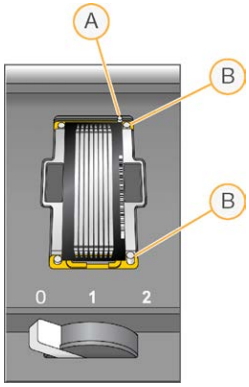
NOTE
Do not remove or replace the flow cell gaskets during this step.

3 Place the used flow cell on the flow cell holder with the inlet and outlet ports facing ***down*** and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

4 Gently slide the flow cell towards the top and right guide pins until it stops.

NOTE
Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.

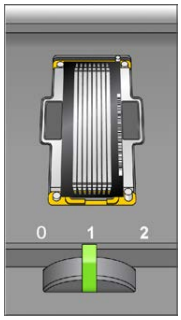
Figure 25 Flow Cell Positioned Against Top and Right Guide Pins



A Top guide pin
B Right guide pins

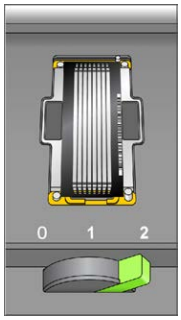
5 Slowly move the flow cell lever to position 1, which engages the vacuum and secures the flow cell into position. When the flow cell lever is blinking green, the vacuum is engaged. If the lever is not green, see *Possible Run Setup Problems* on page 129.

Figure 26 Flow Cell Lever in Position 1



6 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2 (far-right). When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 27 Flow Cell Lever in Position 2



7 Make sure that the **Vacuum Engaged** checkbox is selected on the load prime flow cell screen, and then select **Next**.

Confirm Proper Flow

Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 2 (laboratory-grade water) from the drop-down list.

CAUTION
Use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

2 Confirm the following default values:
- Volume: **125**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

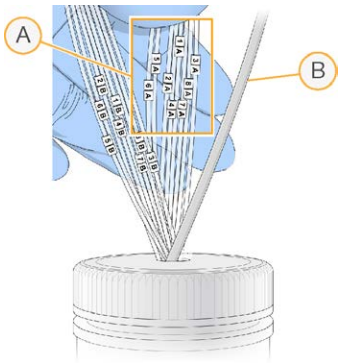
3 Select **Pump**.

4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
If excessive bubbles are present, check the gaskets for obstructions, reduce the aspirate rate to 100, and pump another 125 µl of water to the flow cell. If problems persist, remove the flow cell, repeat the cleaning steps, and reload the flow cell.

Position Tubing and Start Prime

1 Remove the 8 waste tubes for the appropriate flow cell from the waste container. Do not include the 8 tubes for the opposite flow cell or the tube for the condensation pump.

Figure 28 Position Tubing



A Flow cell waste tubes for reagent positions 1–8
B Condensation pump tubing (do not remove)

2 Place waste tubing into an empty 15 ml tube, 1 waste tube per 15 ml tube. Priming waste is collected and measured after the priming step.

3 Select **Start Prime**. The priming screen opens and the priming step begins. Monitor the progress of the priming step from the priming screen.

4 When the priming step is complete, measure the collected waste and confirm that the volume in each tube is 1.75 ml for a total of **14 ml**. The total is calculated as follows:
- 250 µl for each SBS position except position 2 (250 x 7 = 1.75 ml)
- 1.75 ml for each lane (1.75 x 8 = 14 ml)

5 Record the results on the lab tracking form.

6 Return the waste tubing to the waste container before proceeding.

7 Select **Next**.

Load a Flow Cell

Steps to load the clustered flow cell include removing the priming flow cell, cleaning the flow cell holder, cleaning the flow cell, loading the flow cell, and confirming proper flow.

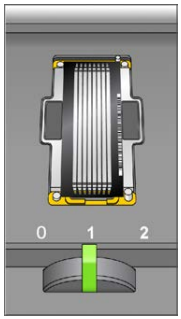
User-Supplied Consumables

- Lens cleaning tissue
- 70% ethanol or alcohol wipes
- Low-lint lab tissue
- One pair of plastistats

Remove the Used Flow Cell

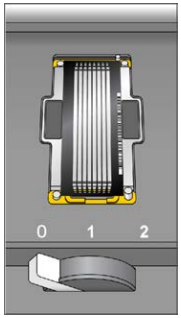
1 Slowly move the flow cell lever to position 1 to disengage the manifolds.

Figure 29 Flow Cell Lever in Position 1



2 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

Figure 30 Flow Cell Lever in Position 0



3 Lift the used flow cell from the flow cell holder.

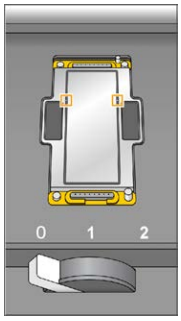
Clean the Flow Cell Holder

1 Put on a new pair of powder-free latex gloves.

2 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

Figure 31 Inspect Vacuum Holes



3 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Clean the Flow Cell

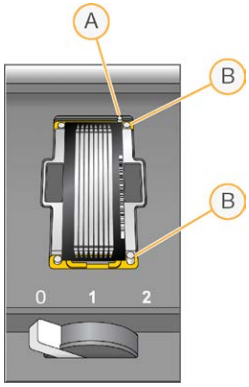
1. Remove the flow cell from the flow cell container using a pair of plastistats.
2. Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.
3. Fold an alcohol wipe to approximately the size of the flow cell.
4. Hold the edges of the clustered flow cell with 2 fingers. Make sure that the inlet and outlet ports are facing ***up***.
5. Wipe each side of the flow cell with a single sweeping motion. Repeat, refolding the alcohol wipe with each pass, until the flow cell is clean.
6. Dry the flow cell using a dry lens cleaning tissue.
7. Protect the flow cell from dust until you are ready to load it onto the instrument.

Load the Sequencing Flow Cell

NOTE
Do not replace the manifold gaskets. Replace the manifold gaskets after the sequencing run is complete and before the maintenance wash.

1. Place the flow cell on the flow cell holder with the inlet and outlet ports facing ***down*** and the barcode on the right. Make sure that the arrow on the left edge of the flow cell points towards the instrument.
2. Gently slide the flow cell towards the top and right guide pins until it stops.

Figure 32 Flow Cell Positioned Against Top and Right Guide Pins

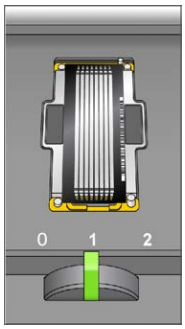


A Top guide pin
B Right guide pins

NOTE
Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.

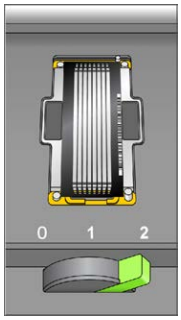
3 Slowly move the flow cell lever to position 1, which engages the vacuum and secures the flow cell into position. When the flow cell lever is blinking green, the vacuum is engaged. If the lever is not green, see *Possible Run Setup Problems* on page 129.

Figure 33 Flow Cell Lever in Position 1



4 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 34 Flow Cell Lever in Position 2



5 Make sure that the **Vacuum Engaged** checkbox is selected on the Load Sequencing Flow Cell screen.

Confirm Proper Flow

Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 5 from the drop-down list.

2 Enter the following default values:
- Volume: **250**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

3 Select **Pump**.

4 Visually inspect the flow cell for bubbles passing through the lanes or leaks near the manifolds.
If excessive bubbles are present, check the manifold gaskets for obstructions and repeat the process using solution 6 to avoid depleting position 5. Reduce the aspirate rate to 100, and pump another 250 µl to the flow cell.

5 Select **Next**. Make sure that the flow cell lever is green, and then close the flow cell compartment door.

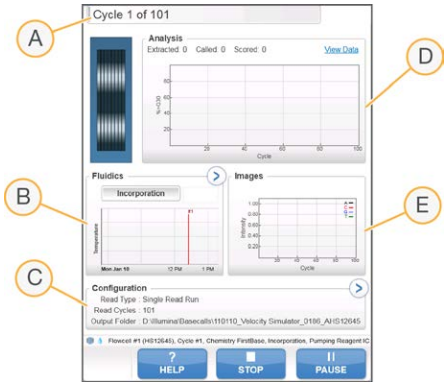
6 Confirm that the checkboxes **Vacuum Engaged** and **Door Closed** are selected, and then select **Next**.

7 Select **Start** to start the sequencing run.

Monitor the Run

Monitor run metrics on the run overview screen, fluidics, and imaging.

Figure 35 Run Overview Screen



A **Progress bar**—Use the progress bar to monitor how many cycles have been completed.
B **Fluidics graph**—Expand the fluidics section to monitor chemistry steps.
C **Run Configuration**—Review parameters of current run.
D **Analysis graph**—Use the analysis graph to monitor quality scores by cycle.
E **Images graph**—Use the images graph to monitor intensities by cycle.

First Base Report

If you selected the **Confirm First Base** option during run setup, the first base confirmation dialog box opens automatically after imaging of the first cycle is complete. The run pauses at this step.

1 Review the First Base Report from the confirmation dialog box.

2 If the results are satisfactory, select **Continue**.

Post-Run Procedures

When the run is complete, unload and weigh reagents, and then perform an instrument wash. For more information, see *Post-Run Procedures* on page 97.

Prepare Reagents for Read 2

Before the completion of Read 1 and any index reads, prepare reagents for Read 2 resynthesis and fresh ICB for Read 2.

For reagent preparation instructions, see the *TruSeq PE Cluster Kit v3 Reagent Prep Guide (part # 15023336)*. This guide includes instructions for preparing sequencing reagents provided in the TruSeq Dual Index Sequencing Primer Box.

NOTE
For optimal performance, Illumina recommends preparing fresh ICB (Incorporation Mix) for Read 2.

Load Reagents for Read 2

After completion of Read 1 and any index reads, load paired-end reagents for Read 2 resynthesis and freshly prepared ICB for Read 2.

Load Paired-End Reagents

1. Record the weight of each reagent on the lab tracking form.
2. Make sure that the paired-end rack is not in use on the opposite flow cell for Read 2 resynthesis, Index 1 (i7) Read preparation, or Index 2 (i5) Read preparation.
3. Raise the sippers for the paired-end reagent rack using the following motion:
   a. Pull the handle towards you and raise the handle.
   b. Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.
4. Slide the reagent rack out of the reagent compartment using the rack handle.
5. Remove the caps from each reagent tube.
6. Place each of the reagent tubes onto the rack in the associated numbered positions.

Table 9 Paired-End Reagent Positions

Position	Reagent	Description
10	RMR	Resynthesis Mix
11	LMX2	Linearization Mix 2
12	BMX	Blocking Mix
13	AMX2	Amplification Mix 2
14	APM2	AMX2 Premix
15	AT2	100% Formamide
16	HP7 or HP11	Read 2 Sequencing Primer
18	HP3	Denaturation Solution
19	HT2	Wash Buffer

NOTE
For dual-indexed paired-end runs, RMR is loaded with indexing reagents before starting the run. For single-indexed or non-indexed runs, RMR is loaded with paired-end reagents.

7 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

8 Lower the sippers into the paired-end reagent tubes as follows:
   a Pull the handle towards you and lower the handle.
   b Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.
   c Release the handle into the slot on the bottom end of the groove.

Load ICB for Read 2

1 Record the weight of the reagent on the lab tracking form.

2 Raise the sippers for the sequencing reagent rack using the following motion:
   a Pull the sipper handle towards you and raise the sipper handle.
   b Release the sipper handle into the slot on top end of the groove. Make sure that the sipper handle rests securely in the slot.

3 Slide the reagent rack out of the reagent compartment.

4 Remove the existing ICB reagent bottle from position 1 of the reagent rack and remove the funnel cap from the bottle.

5 Place the funnel cap on the new bottle of ICB and load the bottle in position 1. Make sure that the conical end of the bottle rests in the indentation on the base of the rack.

6 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

7 Lower the sippers into the sequencing reagent bottles as follows:
   a Pull the sipper handle towards you and lower the sipper handle.
   b Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
   c Release the sipper handle into the slot on the bottom end of the groove.

8 Close the reagent compartment door, and then select **Next** to resume the run.

Perform a Rapid Run

Chapter 4

Introduction

Rapid Run mode provides 2 options for the cluster generation step, either on the cBot or on the HiSeq 2500. Clustering on the cBot allows 2 libraries, 1 each lane, on the 2-lane rapid flow cell. After template hybridization and first extension on the cBot, the remainder of the clustering process is performed on the HiSeq.

After reagent preparation, run setup steps include entering run parameters, loading and priming reagents, loading the flow cell, and performing a fluidics check. If clustering is performed on the HiSeq 2500, the step to prime reagents is omitted in the run setup steps.

Visit the HiSeq 2500 specifications page on the Illumina website for information about run duration and other performance specifications.

Rapid Run Sequencing Consumables

Rapid Run Kit Name	Description
HiSeq Rapid SBS Kit v2 or TruSeq Rapid SBS Kit (v1)	Contains SBS reagents used on the HiSeq 2500 and funnel caps for SBS reagent bottles.
HiSeq Rapid PE Cluster Kit v2 or HiSeq Rapid SR Cluster Kit v2 or TruSeq Rapid PE Cluster Kit (v1) or TruSeq Rapid SR Cluster Kit (v1)	Contains clustering and indexing reagents used on the HiSeq 2500 and a set of flow cell gaskets. The PE versions of the cluster kits include paired-end reagents used on the HiSeq 2500.

Reagent Preparation Steps

Before setting up the run, prepare SBS reagents, indexing reagents, and paired-end reagents.

- For SBS reagent preparation, see the appropriate guide:
  - *HiSeq Rapid SBS Kit v2 Reagent Prep Guide (part # 15058772)*
  - *TruSeq Rapid SBS Kit Reagent Prep Guide (200 Cycles) (part # 15036501)*
  - *TruSeq Rapid SBS Kit Reagent Prep Guide (50 Cycles) (part # 15036502)*
- For indexing and paired-end reagent preparation, see the appropriate guide:
  - *HiSeq Rapid Cluster Kit v2 Reagent Prep Guide (part # 15059131)*

- *TruSeq Rapid PE Cluster Kit Reagent Prep Guide (part # 15038861)*
- *TruSeq Rapid SR Cluster Kit Reagent Prep Guide (part # 15038860)*

Prepare all reagents before setting up the run. When prompted by the control software, load all reagents. When using rapid run chemistry, there is no need to return to the instrument during the run to load reagents.

Rapid Run Sequencing Workflow

Prepare all reagents for the run and prepare the library template.

For reagent preparation information, see *Reagent Preparation Steps* on page 70.

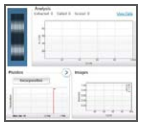
Using the control software, perform a volume check and enter run parameters.

For on-instrument clustering—Load all reagents for the run and the prepared library template.

For cluster generation on the cBot—Load all reagents for the run.

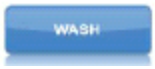
With a used flow cell on the instrument, confirm proper flow.

For cluster generation on the cBot—Prime SBS reagents and measure priming waste.



Start the sequencing run. After cycle 1, inspect the first base report (optional setting), and then continue Read 1.

The sequencing run continues through the PE turn and Read 2 with no intervention necessary.



When the run is complete, unload and weigh reagents. Perform a post-run water wash.

Run Types for Rapid Run Chemistry

The following tables show types of sequencing runs and the number of possible cycles for each read when using rapid run chemistry. Use this information as a reference when setting up the run.

Table 10 HiSeq Rapid SBS Kit v2

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 251	--	--	--	≤ 251
Single-Read, Single-Indexed	≤ 251	7 [1] 8 [2]	--	--	≤ 258 [1] ≤ 259 [2]
Single-Read, Dual-Indexed	≤ 251	8	8	--	≤ 267
Paired-End, Non-Indexed	≤ 251	--	--	≤ 251	≤ 502
Paired-End, Single-Indexed	≤ 251	7 [1] 8 [2]	--	≤ 251	≤ 509 [1] ≤ 510 [2]
Paired-End, Dual-Indexed	≤ 251	8	7 + 8 [3]	≤ 251	≤ 525

Table 11 TruSeq Rapid SBS Kit (v1)

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 101	--	--	--	≤ 101
Single-Read, Single-Indexed	≤ 101	7 [1] 8 [2]	--	--	≤ 108 [1] ≤ 109 [2]
Single-Read, Dual-Indexed	≤ 101	8	8	--	≤ 117
Paired-End, Non-Indexed	≤ 101	--	--	≤ 101	≤ 202
Paired-End, Single-Indexed	≤ 101	7 [1] 8 [2]	--	≤ 101	≤ 209 [1] ≤ 210 [2]
Paired-End, Dual-Indexed	≤ 101	8	7 + 8 [3]	≤ 101	≤ 225

[1] Number of cycles for single-indexed libraries
[2] Number of cycles for dual-indexed libraries
[3] Index 2 Read of a paired-end dual-indexed run includes 7 additional chemistry-only cycles

Pre-Run Volume Check

From the Welcome screen, select **Sequence** | **New Run**. The Volume Check screen opens.

Volume Check Screen

1. When prompted by the software to perform a volume check, select **Yes**.
2. Place waste tubes 1, 2, 3, 6, 7, and 8 for the current flow cell in a 1 liter bottle filled with deionized water. Placing the tubes in deionized water prevents damage to the reagent pumps.
3. Load laboratory-grade water into all 8 SBS positions, 10 positions on the paired-end rack, and the library position for the current flow cell.
4. Close the loading station.
5. Select the **Water loaded and template loading station closed** checkbox.
6. Select **Next**.
7. Make sure that a used rapid flow cell is loaded on the instrument. Enter the ID of the used flow cell.
8. Select **Next**.
9. Select **Pump** to confirm flow.
10. Place tubes 4 and 5 into separate empty 15 ml conical tubes.
11. Select **Next**. The volume check begins.
    When the volume check is complete, the expected volume is 9.5 ml ±10% for each tube.
12. Return all tubes to the waste bottle.
13. Select **Next**.

Enter Run Parameters

From the Welcome screen, select **Sequence** | **New Run**.

The control software interface guides you through the steps to set up the run. Run setup steps are organized in 3 tabs: Run Configuration, Pre-Run Setup, and Initiate Run.

- Run configuration screens contain drop-down lists, checkboxes, or text fields for run parameters. Use the hand-held barcode scanner to scan the flow cell or reagent kit ID, or enter the ID using the touch screen keyboard. The keyboard icon is located to the right of the text fields. 
- Select **Next** to move to the next screen, or select **Back** to return to the previous screen.
- At any time during the run setup steps, select **Cancel** to exit run setup and return to the Welcome screen.

Integration Screen

The Integration screen provides the option to connect the run to BaseSpace.

To connect to BaseSpace, do the following:

1. Select **BaseSpace**.
2. Select from the following BaseSpace options:
   - **Storage and Analysis**—Sends run data to BaseSpace for remote monitoring and data analysis. A sample sheet is required with this option.
   - **Run Monitoring Only**—Sends only InterOp files to BaseSpace, which allows remote monitoring of the run.
3. Log on to BaseSpace using your MyIllumina account email and password.
4. Select **Next**.

To proceed without connecting to BaseSpace, do the following:

1. Select **None**.
2. Select **Next**.

Storage Screen

1. Select the **Save to an output folder** checkbox, and select **Browse** to navigate to a preferred network location. If the run is connected to BaseSpace for storage and

analysis, this field is optional.

2 Select **Zip BCL files** to reduce required storage space. If the run is connected to BaseSpace, the **Zip BCL files** option is selected by default.

NOTE
The **Bin Q-Scores** setting is enabled by default to reduce required storage space. This setting groups quality scores over a wider range of values without affecting accuracy or performance.

3 Select from the following Save Auxiliary Files options:
- **Save All Thumbnails**—Saves all thumbnails images. A thumbnail is a sampling of images from many tiles in each column of tiles, or swath, combined in 1 thumbnail image.
- **Save Tile Thumbnails**—Saves tile thumbnails. Tile thumbnails represent a single tile rather than a sampling of tiles in a swath.

4 Select **Next**.

Flow Cell Setup Screen

The Flow Cell Setup screen records information about the flow cell used for the run.

1 Select a Reagent Kit Type, either **TruSeq Rapid v1** or **HiSeq Rapid v2**.

2 Scan the flow cell barcode or enter the flow cell ID (barcode number) of the flow cell to be sequenced. The flow cell ID is used to determine flow cell type and reagent compatibility.

3 Confirm that the flow cell type is correct, either **TruSeq Rapid Flow Cell v1** or **HiSeq Rapid Flow Cell v2**. The flow cell type is automatically selected based on the flow cell ID.

4 Enter an experiment name. The experiment name appears on each screen to help identify the run in progress.

5 Enter a user name.

6 Select **Next**.

Advanced Screen

1 [Optional] Select the **Confirm First Base** checkbox.

A first base report is generated automatically for each run. Selecting this option opens the first base report before proceeding with the run.

2 [Optional] From the **Align to PhiX** checkboxes, clear the checkbox for lanes that do not contain PhiX.
By default, all lanes are selected for alignment by Real-Time Analysis.
Alternatively, select lanes on the flow cell image to add or remove lanes for PhiX alignment.

NOTE
A dedicated control lane is not required with HCS v2.2 and RTA v1.18. Therefore, the option to assign a control lane is not available with this software configuration.

3 [Optional][For TruSeq Rapid v1] Select **Keep Intensity Files** for later reanalysis or custom processing.
By default, this option is not selected. Saving intensity files is not required for on-instrument analysis. Enabling this option significantly increases the size of the data output folder.

4 Select **Next**.

Recipe Screen

A recipe is generated automatically from the information entered on the Recipe screen.

1 Select from the following Index Type options:
- **No Index**—Performs a non-indexed single-read or paired-end run.
- **Single Index**—Performs a single-read or paired-end run with 1 indexing read.
- **Dual Index**—Performs a single-read or paired-end run with 2 indexing reads.
- **Custom**—Performs a single-read or paired-end run with a custom number of cycles for index reads.

2 If the Dual Index or Custom option is specified, select a Flow Cell Format, either **Single Read** or **Paired End**.

3 Enter the number of cycles for Read 1 and Read 2, if applicable.

NOTE
The number of cycles performed in a read is 1 more cycle than the number of cycles analyzed. For example, to perform 100 cycles for Read 1, enter 101.

For the **Custom** indexing option, enter the number of cycles for index reads. Read lengths do not need to be identical.

4 Confirm the following chemistry settings. These fields are auto-populated depending on the selected reagent kit type and flow cell format option.
   a **SBS**: **TruSeq Rapid SBS Kit v1** or **HiSeq Rapid SBS Kit v2**
   b **Cluster Kit**: **TruSeq Rapid PE Cluster Kit v1**, **TruSeq Rapid SR Cluster Kit v1**, **HiSeq Rapid PE Cluster Kit v2**, or **HiSeq Rapid SR Cluster Kit v2**

5 [Optional] Select the **Use Existing Recipe** checkbox to use a custom recipe. Otherwise, allow the software to create the recipe from run parameters entered.

Sample Sheet Screen

Sample sheets are optional unless you are using BaseSpace to perform data analysis, performing an indexing run, or planning to monitor demultiplexing performance using Sequencing Analysis Viewer. For more information, see the *Sequencing Analysis Viewer User Guide (part # 15020619)*.

1 Select from the following options to specify the clustering method:
   - Select **On-Board Cluster Generation** to perform clustering on-instrument.
   - If clustering started on the cBot, select **Template Hybridization on cBot**.

2 Select **Next**.

3 In the Sample Sheet field, select **Browse** and navigate to the sample sheet location.

4 Select **Next**.

Reagents Screen

The Reagents screen records information about reagent kits used for the run. The reagent kit ID (barcode number beginning with **RGT**) is used to determine reagent kit type and run mode compatibility.

1 Scan or enter the SBS reagent kit ID.

2 For paired-end runs, scan or enter the reagent kit ID for the cluster kit.

3 Select the SBS reagent kit for the run:
   - [For HiSeq Rapid SBS Kit v2] Select **500 Cycles** for a 500 cycle kit. Cycles remaining defaults to 525 cycles remaining.
   - Select **200 Cycles** for a 200 cycle kit. Cycles remaining defaults to 225.
   - Select **50 Cycles** for a 50 cycle kit. Cycles remaining defaults to 74.

- Select **Custom** for a partial kit or multiple 50-cycle kits. In the Cycles Remaining field, enter the number of SBS cycles that reagents are expected to last.

NOTE
For partial kits, the software counts down the number of cycles entered. When the cycles are low, the software prompts you to load fresh reagents.

4 Select **Next**.

Review Screen

1 Review the run parameters on the Review screen.

2 Select **Next** to proceed or select **Back** to change parameters.

Load and Prime Reagents

After entering run parameters, load SBS, clustering, indexing, and paired-end reagents for the run, and then prime reagents through the fluidics system. The software guides you through these steps in a series of screens on the Pre-Run Setup tab.

Illumina-Supplied Consumables

- Eight funnel caps

User-Supplied Consumables

- 250 ml bottle (Corning, catalog # 430776)
- 15 ml conical tubes (Corning, catalog # 430052)
- Laboratory-grade water

NOTE
To prepare for the post-run rinse at the end of a sequencing run, load 25 ml PW1 or laboratory-grade water in position 2.
The post-run rinse does ***not*** replace the post-run instrument wash.

Load SBS and Clustering Reagents

Make sure that SBS reagents are ready to load onto the instrument.

Illumina-Supplied Consumables

- Eight funnel caps

User-Supplied Consumables

- One 250 ml bottle (Corning, catalog # 430776)
- One flip-top Eppendorf tube per flow cell (1.5 ml or 1.7 ml). Do not use tubes with screw caps.

Procedure

1. Record the weight of each reagent on the lab tracking form.
2. Open the reagent compartment door.
3. Raise the sippers for the sequencing reagent rack using the following motion:

a Pull the handle towards you and then raise the handle.
b Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.

4 Slide the SBS reagent rack out of the reagent compartment.

5 Remove the cap from each reagent bottle and replace it with a funnel cap. Replace the cap on the bottle of CRM last, and then replace your gloves.

6 Place each SBS reagent bottle onto the rack in the associated numbered position. Make sure that the conical end of the bottle rests in the indentation on the base of the rack.

Table 12 HiSeq Rapid v2 SBS Reagent Positions

Position	Reagent	Description
1	IMT	Incorporation Master Mix
2	PW1	25 ml PW1 or laboratory-grade water
3	USM	Universal Scan Mix
4	PW1	25 ml PW1 or laboratory-grade water
5	USB	Universal Sequencing Buffer
6	USB	Universal Sequencing Buffer
7	CRM	Cleavage Reagent Master Mix
8	CWM	Cleavage Wash Mix

Table 13 TruSeq Rapid (v1) SBS Reagent Positions

Position	Reagent	Description
1	IMM	Incorporation Master Mix
2	PW1	25 ml PW1 or laboratory-grade water
3	SRM	Scan Reagent Master Mix
4	PW1	25 ml PW1 or laboratory-grade water
5	USB	Universal Sequencing Buffer
6	USB	Universal Sequencing Buffer
7	CRM	Cleavage Reagent Master Mix
8	PW1	25 ml PW1 or laboratory-grade water

7 Load 25 ml PW1 or laboratory-grade water in the following positions:
- [For HiSeq Rapid SBS Kit v2] Positions 2 and 4.
- [For TruSeq Rapid SBS Kit] Positions 2, 4, and 8.

8 Slide the SBS rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

9 Select the **PW1 (25 ml) loaded** checkbox.

10 Slide the PE reagent rack out of the reagent compartment.

11 Remove the caps from each reagent tube and place the tube onto the rack in the associated numbered position.

Table 14 Single-Read Flow Cell

Position	Reagent	Description
10	PW1	10 ml PW1 or laboratory-grade water
11	PW1	10 ml PW1 or laboratory-grade water
12	PW1	10 ml PW1 or laboratory-grade water
13	AMS	Fast Amplification Mix
14	FPM	Fast Premix
15	FDR	Fast Denaturation Reagent (contains formamide)
16	HP9*	i5 Index Primer
17	HP12*	i7 Index Primer
18	HP10	Read 1 Primer
19	FLS	Fast Linearization Solution

*HP9 is required only for dual-indexed runs. HP12 is required for all indexing options. If HP9 and HP12 are not used, load a 15 ml conical tube filled with 10 ml PW1 or laboratory-grade water in position 16 for HP9 and position 17 for HP12.

Table 15 Paired-End Flow Cell

Position	Reagent	Description
10	FRM	Fast Resynthesis Mix
11	FLM2	Fast Linearization Mix 2 (Read 2)
12	FLM1	Fast Linearization Mix 1 (Read 1)
13	AMS	Fast Amplification Mix
14	FPM	Fast Premix
15	FDR	Fast Denaturation Reagent (contains formamide)
16	HP11	Read 2 Primer
17	HP12*	i7 Index Primer
18	HP10	Read 1 Primer
19	PW1	10 ml PW1 or laboratory-grade water

*HP12 is required only for indexed runs. If HP12 is not used, load a 15 ml conical tube filled with 10 ml PW1 or laboratory-grade water in position 17.

12 Add 10 ml PW1 or laboratory-grade water to 15 ml conical tubes in the following

positions:

- Paired-end run—Position 19
  Non-indexed—Position 17
- Single-read run—Positions 10, 11, and 12
  Non-indexed—Positions 16 and 17

13 Slide the PE rack into the reagent compartment, aligning the racks with the raised guide on the floor of the compartment.

14 Lower the sippers into the sequencing reagent bottles as follows:
   a Pull the sipper handle towards you and then lower the sipper handle.
   b Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
   c Release the sipper handle into the slot on the bottom end of the groove.

Load Template

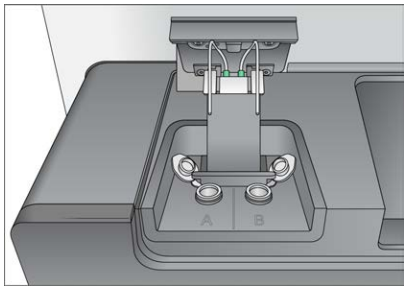
Load the library template for clustering on the instrument. For library preparation instructions, see *Denaturing and Diluting Libraries for the HiSeq and GAIIx (part # 15050107)*.

NOTE
If the cBot was used for clustering, place 2 Eppendorf tubes filled with 1 ml deionized water into the loading station instead of using the following instructions.

1 Add 420 µl prepared library template in a 1.5 ml or 1.7 ml Eppendorf tube.

2 Load the template into the loading station, as follows:
   a Lift the door of the loading station.
   b Remove the Eppendorf tube containing water and replace it with the Eppendorf tube containing 420 µl of prepared library template.
   c Secure the lids under the bar behind the tubes to avoid interfering with the sippers.

Figure 36 Loading Station



NOTE
The liquid remaining in the tube after the run is highly diluted and not suitable for further use.

d Slowly close the loading station door, making sure that the sippers are properly aligned with the Eppendorf tubes when the lid is closed.

3 Select the **Template loaded and template loading station closed** checkbox.

4 Select **Next**.

Prime Reagents

NOTE
Prime reagents only if the HiSeq or TruSeq Rapid Duo Sample Loading Kit was used to perform template hybridization on the cBot. Otherwise, skip reagent priming and proceed to *Load a Flow Cell* on page 90.

Steps for priming reagents include cleaning the flow cell holder, loading a used flow cell, confirming proper flow, and then starting the prime.

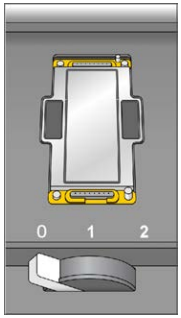
Clean the Flow Cell Holder

1 Open the flow cell compartment door.

CAUTION
Do not place fluids on the flow cell compartment door or on the flow cell stage when the door is open. Spills in this area can damage the instrument.

2 Make sure that the flow cell lever is in the OFF position.

Figure 37 Flow Cell Lever in Position 0



3 Put on a new pair of powder-free latex gloves.

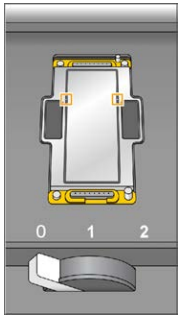
4 If the flow cell from a previous run is present, remove it and set aside in a tube of storage buffer or laboratory-grade water to keep it from drying out. It can be used to confirm proper flow before loading the clustered flow cell.

5 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

6 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Figure 38 Vacuum Hole Locations



Load a Priming Flow Cell

NOTE
Use a used flow cell to prime reagents. Do not use the flow cell that you want to sequence.

1 Rinse a used flow cell with laboratory-grade water. Dry it with lens cleaning tissue or lint-free tissue.

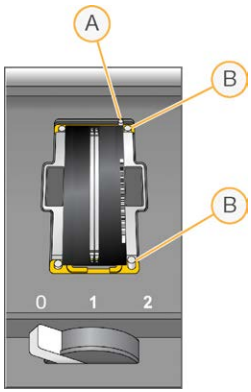
2 Clean the flow cell using alcohol wipes and lens cleaning tissue.

NOTE
Do not remove or replace the manifold gaskets.

3 Place the used flow cell on the flow cell holder with the inlet and outlet ports facing ***down*** and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

4 Gently slide the flow cell towards the top and right guide pins until it stops.

Figure 39 Flow Cell Positioned Against Top and Right Guide Pins

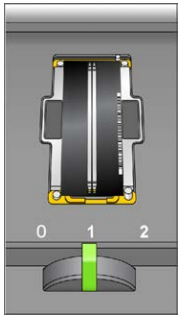


A Top Guide Pin
B Right Guide Pins

NOTE
Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.

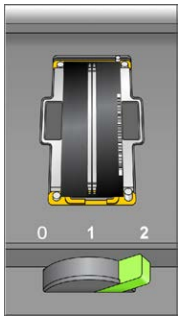
5 Slowly move the flow cell lever to position 1 to engage the vacuum and secure the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 40 Flow Cell Lever in Position 1



6 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2 (far-right). When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 41 Flow Cell Lever in Position 2



7 Enter the flow cell ID.

NOTE
You can use a TruSeq Rapid flow cell or a HiSeq Rapid v2 flow cell for priming.

8 Make sure that the **Vacuum Engaged** checkbox is selected on the load prime flow cell screen, and then select **Next**.

Confirm Proper Flow

After the used flow cell is loaded, check for proper flow. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 2 (laboratory-grade water) from the drop-down list.

CAUTION
Use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

2 Confirm the following default values:
- Volume: **250**
- Aspirate Rate: **1500**
- Dispense Rate: **2000**

3 Select **Pump**.

4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
If excessive bubbles are present, check the gaskets for obstructions, reduce the aspirate rate to 1000, and pump another 250 µl of water to the flow cell. If problems persist, remove the flow cell, repeat the cleaning steps, and reload the flow cell.

Position Tubing and Prime Reagents

1 Loosen and remove the waste tubes for the appropriate flow cell from the waste container. Do not include the 8 tubes for the opposite flow cell or the tube for the condensation pump.

2 Place tube 4 and tube 5 into separate 15 ml tubes.

3 Place tubes 1, 2, 3, 6, 7, and 8 into a bottle containing laboratory-grade water.

4 Select **Next**.

5 Select **Start Prime** to start priming. You can monitor the progress of the priming from the Prime screen.

6 When priming is complete, measure the collected priming waste and confirm that the volume is 2.5 ml ±10%, which is 500 µl per reagent, per lane. Record the results on the lab tracking form.

7 Return tubes 4 and 5 to the waste container before proceeding. Leave waste tubes 1, 2, 3, 6, 7, and 8 in the bottle containing laboratory-grade water.

8 Select **Next**.

Load a Flow Cell

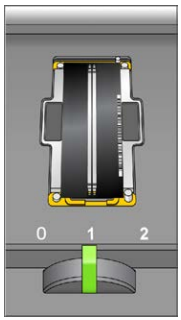
The next step is to remove the used flow cell and load the flow cell that you want to sequence.

NOTE
If the cBot was used for clustering, load the clustered flow cell. For on-instrument clustering, load a new flow cell.

Remove the Used Flow Cell

1 Open the flow cell compartment door.

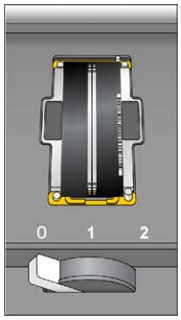
Figure 42 Flow Cell Lever in Position 1



2 Slowly move the flow cell lever to position 1 to disengage the manifolds.

3 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

Figure 43 Flow Cell Lever in Position 0



4 Lift the used flow cell from the flow cell holder.

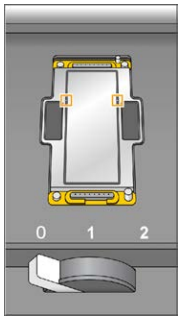
Clean the Flow Cell Holder

1 Put on a new pair of powder-free latex gloves.

2 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

Figure 44 Inspect Vacuum Holes



3 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Clean the Flow Cell

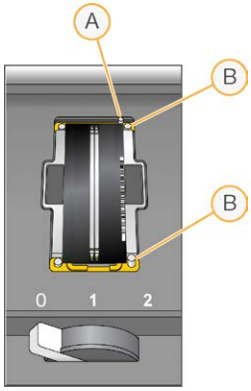
1. Remove the flow cell from the flow cell container using a pair of plastistats.
2. Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.
3. Fold an alcohol wipe to approximately the size of the flow cell.
4. Hold the edges of the clustered flow cell with 2 fingers. Make sure that the inlet and outlet ports are facing ***up***.
5. Wipe each side of the flow cell with a single sweeping motion. Repeat, refolding the alcohol wipe with each pass, until the flow cell is clean.
6. Dry the flow cell using a dry lens cleaning tissue.
7. Protect the flow cell from dust until you are ready to load it onto the instrument.

Load the Sequencing Flow Cell

NOTE
Do not replace the manifold gaskets. Replace the manifold gaskets after the sequencing run is complete and before the maintenance wash.

1. Place the flow cell on the flow cell holder with the inlet and outlet ports facing ***down*** and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.
2. Gently slide the flow cell towards the top and right guide pins until it stops.

Figure 45 Flow Cell Positioned Against Top and Right Guide Pins

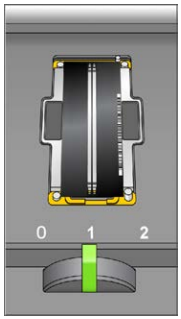


A Top Guide Pin
B Right Guide Pins

NOTE
Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.

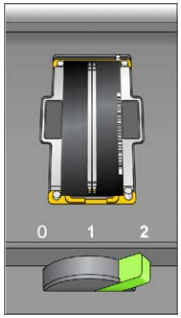
3 Slowly move the flow cell lever to position 1 to engage the vacuum and secure the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 46 Flow Cell Lever in Position 1



4 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 47 Flow Cell Lever in Position 2



5 Make sure that the **Vacuum Engaged** checkbox is selected on the load sequencing flow cell screen.

Confirm Proper Flow

1 Select solution 5 (USB) from the drop-down list.

2 Make sure that the following default values are entered:
- Volume: **250**
- Aspirate Rate: **1500**
- Dispense Rate: **2000**

3 Make sure that waste outlet tubes 1, 2, 3, 6, 7, and 8 are in a bottle of clean water and that tubes 4 and 5 are in the waste container.

4 Select **Pump**.

5 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
If excessive bubbles are present, check the manifold gaskets for obstructions, and repeat the process.
   a Select solution 6 (USB) to avoid depleting USB from position 5.
   b Reduce the aspirate rate to 1000, and pump another 250 µl of USB to the flow cell.

6 After you have confirmed proper flow, select **Next** to proceed.

7 Make sure that the flow cell lever is green, and then close the flow cell compartment door.

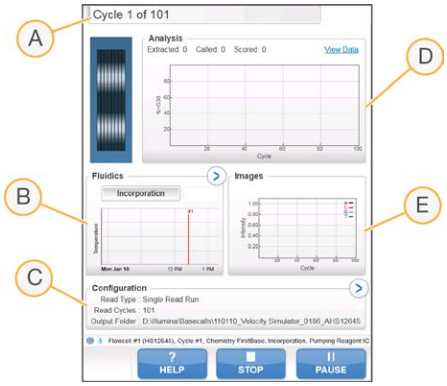
8 Confirm that the **Vacuum Engaged** and **Door Closed** checkboxes are selected, and then select **Next**.

9 Select **Start** to start the sequencing run.

Monitor the Run

Monitor run metrics on the run overview screen, fluidics, and imaging.

Figure 48 Run Overview Screen



A **Progress bar**—Use the progress bar to monitor how many cycles have been completed.
B **Fluidics graph**—Expand the fluidics section to monitor chemistry steps.
C **Run Configuration**—Review parameters of current run.
D **Analysis graph**—Use the analysis graph to monitor quality scores by cycle.
E **Images graph**—Use the images graph to monitor intensities by cycle.

First Base Report

If you selected the **Confirm First Base** option during run setup, the first base confirmation dialog box opens automatically after imaging of the first cycle is complete. The run pauses at this step.

1 Review the First Base Report from the confirmation dialog box.

2 If the results are satisfactory, select **Continue**.

Post-Run Procedures

When the run is complete, unload and weigh reagents, and then perform an instrument wash. For more information, see *Post-Run Procedures* on page 97.

Post-Run Procedures

Chapter 5

Introduction

Post-run procedures include the removal and weighing of reagents, and an instrument wash. A water wash is required after each run, with the option after a high output run to perform a maintenance wash instead. Illumina recommends a maintenance wash.

Regular instrument washes ensure continued performance in the following ways:

- Flush any remaining reagents and sample from the fluidics tubing and sippers.
- Prevent salt accumulation and crystallization in the fluidics tubing and sippers.
- Prevent cross-contamination from the previous run, including cross-contamination after switching modes.

NOTE

A water wash performed at the end of a run washes the system and checks fluidics. When setting up a run, the software confirms that a water wash or maintenance wash has been performed within the last 24 hours. A maintenance wash is required every 10 days. When 10 days elapse after the last maintenance wash, the software prompts you to perform a maintenance wash.

Unload and Weigh Reagents

1. Open the reagent compartment door.
2. Raise the sippers for the appropriate SBS rack and paired-end rack using the following motion:
   a. Pull the sipper handle outward.
   b. Raise the sipper handle while pulling it outward.
   c. Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.
3. Slide the reagent rack out of the reagent compartment using the rack handle.
4. Remove each bottle from the reagent rack and record the weight on the lab tracking form.
   Visit the HiSeq 2500 support page on the Illumina website to download an interactive lab tracking form.
5. [For Rapid Run mode] Remove tubes from the library loading station.

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit at support.illumina.com/sds.html.

Perform a Maintenance Wash

A maintenance wash is required every 10 days or when switching between high output and rapid modes, and is an option after a high output run. Illumina recommends performing a maintenance wash after a high output run.

The Load Gasket screen opens with a maintenance wash every 10 days and when switching from rapid mode to a high output mode. Replace the 10-port gasket in the front manifold and the 8-port gasket in the back manifold before proceeding to the wash, even if this screen does not appear.

A maintenance wash consists of washing the system with Tween 20 and ProClin 300.

User-Supplied Consumables

- Ethanol wipes
- 8 bottles, 250 ml (Corning, catalog # 430776)
- 10 tubes, 15 ml (Corning, catalog # 430052)
- [For Rapid Run mode] 1 Eppendorf tube per flow cell for washing the loading station
- Laboratory-grade water
- Tween 20 (Sigma-Aldrich, catalog # P7949)
- ProClin 300 (Sigma-Aldrich, catalog # 48912-U)

Prepare Wash Solution

Prepare 5 liters of maintenance wash solution for use with 1 instrument. The solution can be used up to 3 times and stored for up to 30 days at room temperature.

NOTE
Dispose of wash solution in accordance with the governmental safety standards for your region.

1. Prepare 250 ml of 10% Tween 20 by combining the following volumes, adding the water first:
   - Laboratory-grade water (225 ml)
   - Tween 20 (25 ml)
2. Place a stir bar in an empty carboy that is at least 6 liters.
3. Combine the following volumes in the carboy, adding the water first:

- Laboratory-grade water (750 ml)
- 10% Tween 20 (250 ml)
- ProClin 300 (1.5 ml)

These volumes result in approximately 2.5% Tween 20 and 0.15% ProClin 300 solution.

4 Place the carboy onto a stir plate and stir until the solution is thoroughly mixed.

5 Add 4 liters laboratory-grade water to the solution. These volumes result in approximately 0.5% Tween 20 and 0.03% ProClin 300 wash solution.

6 Continue stirring until the solution is thoroughly mixed.

7 Set aside in a closed container at room temperature until you are ready to fill or replenish reagent bottles and tubes with wash solution.

Tween 20 and ProClin 300 Wash

1 From the Welcome screen, select **Wash** | **Maintenance**.

2 [For high output modes] Select **Yes** to wash PE reagent positions when the sequencing run included an indexing read or PE turn. Otherwise, select **No**. Select **Next** to proceed.

3 If you are using fresh wash solution, prepare the wash components as follows:

a Fill 8 SBS bottles with 250 ml wash solution.

b Fill 10 PE tubes with 12 ml wash solution.

c [For Rapid Run mode] Fill Eppendorf tubes with 1.6 ml wash solution and load them into the loading station.

If you are reusing wash solution, replenish the bottles and tubes saved from the previous wash.

4 For fresh wash solution, assign each bottle and tube to a reagent rack position and maintain those positions for each subsequent wash. Otherwise, the wash solution might become contaminated with reagents that were present on the sippers.

5 Load the bottles and tubes onto the instrument in the assigned reagent rack position.

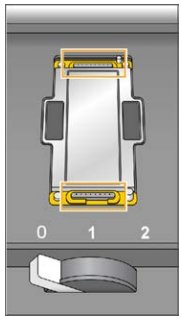
6 Select the **Wash solution loaded and template loading station closed** checkbox.

7 Select **Next**.

8 Remove the flow cell from the flow cell stage and set it aside until you are ready to reload the same flow cell before starting the wash.

9 Wearing a new pair of gloves, apply light pressure to 1 side of the front gasket until the other side lifts. Use tweezers to grasp and remove the gasket. Repeat to remove the rear gasket.

Figure 49 Remove Used Manifold Gaskets



10 Position a new 10-port gasket in the front manifold and a new 8-port gasket in the rear manifold.

11 Reload the flow cell.

12 Make sure that the **Vacuum Engaged** checkbox is selected on the Load Wash Flow Cell screen.

13 Select **Next**.

14 Perform a fluidics check:
   a Select solution 2 from the drop-down list. Accept the default pump values.
   b Select **Pump**.
   c Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds. Extra bubbles are normal for the Tween 20 and ProClin 300 wash, and do not affect delivered volume.

15 Remove the 8 waste tubes for the appropriate flow cell from the waste container. Do not include the 8 tubes for the opposite flow cell, or the tubes from the condensation pump.

16 [For high output modes] Bundle the 8 waste tubes with parafilm, making sure to keep all of the tubes even. Place the bundled tube ends into a 250 ml bottle.

17 [For Rapid Run mode] Place the ends of tubes 4 and 5 into an empty container. Place the end of all of the other tubes into a bottle of clean water to prevent air from being introduced into the syringe pumps.

18 Select **Next** to start the wash.

19 When the wash is complete, select **Return to Start**.

20 Measure the delivered volume.

NOTE
All bottles and tubes are filled to capacity to make sure that the sippers are rinsed. However, the delivered volume for each position varies so the bottles and tubes contain different volumes when the wash is complete.

Positions	High Output Flow Cell Delivered Volume	Rapid Flow Cell Delivered Volume
8 SBS positions	82 ml	29 ml
10 PE positions	76 ml	30 ml
1 library position	Empty	1.2 ml
All positions	19.75 ml per lane	30.1 ml per lane

21 Unwrap the waste tubes and return them to the waste container.

Perform a Water Wash

A water wash is required after each sequencing run. After a high output run, you can perform a maintenance wash instead.

If the instrument has been idle for 1 day or more, perform a water wash before beginning a new sequencing run.

Illumina-Supplied Consumables

- Laboratory-grade water

User-Supplied Consumables

- 8 bottles, 250 ml (Corning, catalog # 430776)
- 10 tubes, 15 ml (Corning, catalog # 430052)
- Laboratory-grade water
- [For Rapid Run mode] 1 Eppendorf flip-top tube for each flow cell

Procedure

1. From the Welcome screen, select **Wash** | **Water**.
2. Select **Yes** to wash paired-end reagent positions. Otherwise, select **No** to wash SBS reagent positions only. Select **Next** to proceed.
3. Load the instrument with laboratory-grade water as follows:
   a. Fill 8 SBS bottles with 20 ml laboratory-grade water.
   b. Fill 10 PE tubes with 10 ml laboratory-grade water.
   c. [For Rapid Run mode] Fill the Eppendorf tube with 1 ml laboratory-grade water.
4. Make sure that a used flow cell is loaded. Load a used flow cell, if necessary. Select **Next**.
5. Perform a fluidics check:
   a. Select solution 2 from the drop-down list. Accept the default pump values.
   b. Select **Pump**.
   c. Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

6 Remove the waste tubing for the appropriate flow cell from the waste container. Do not include the waste tubing for the opposite flow cell, or the tubing from the condensation pump.

7 [For high output modes] Bundle the waste tubing with parafilm, making sure to keep all of the ends even. Place the bundled tubing ends into a 250 ml bottle.

8 [For Rapid Run mode] Place the ends of tubes 4 and 5 into an empty container. Place the end of all of the other tubing into a bottle of clean water to prevent air from being introduced into the syringe pumps.

9 Select **Next** to start the water wash.

Positions	Approximate Run Time
8 SBS positions	20 minutes
8 SBS positions and 10 paired-end positions	60 minutes
[Rapid Run mode] 8 SBS positions, 10 paired-end positions, and 1 library loading position	10 minutes

10 When the wash is complete, measure the delivered volume and record it on the lab tracking form.

Positions	Delivered Volume
8 SBS positions	32 ml
8 SBS positions and 10 paired-end positions	72 ml
[Rapid Run mode] 8 SBS positions, 10 paired-end positions, and 1 library loading position	9.5 ml per lane

11 Unwrap the waste tubing and return the tubing to the waste bottle.

Switch Sequencing Modes

Use the **Mode Select** command from the Welcome screen to switch between high output modes and Rapid Run mode.

Only runs of the same mode can be performed simultaneously. Therefore, mode changes are applied to both flow cell A and flow cell B. If either flow cell is in progress, a mode change is not possible.

A maintenance wash and gasket change are required when switching between run modes. For more information, see *Perform a Maintenance Wash* on page 100.

Switch from High Output to Rapid Run Mode

Switching from a high output mode (HiSeq v4 or TruSeq v3) to Rapid Run mode requires a rapid-mode maintenance wash.

Specification	Rapid Maintenance Wash
Flow Cell Type	Rapid flow cell (2 lanes)
Flow Cell Gasket	10-port gasket and 8-port gasket
Reagents	Tween 20 and ProClin 300
Expected Volumes (ml)	60.2 ml
Time (minutes)	60 minutes

Switch from Rapid Run to High Output Mode

Switching from Rapid Run mode to a high output mode (HiSeq v4 or TruSeq v3) requires a rapid-mode maintenance wash followed by high-output maintenance wash.

Specification	Rapid Maintenance Wash	High Output Maintenance Wash
Flow Cell Type	Rapid flow cell (2 lanes)	High output flow cell (8 lanes)
Flow Cell Gasket	10-port gasket and 8-port gasket	10-port gasket and 8-port gasket
Expected Volumes (ml)	60.2 ml	158 ml
Time (minutes)	60 minutes	130 minutes
Total Mode Change Time	~ 3 hours	

Idle the Instrument

Use the following instructions to prepare the instrument to sit idle for up to 10 days. For durations longer than 10 days, see *Shut Down the Instrument* on page 109.

1. Perform a complete maintenance wash to flush the system fully. For more information, see *Perform a Maintenance Wash* on page 100.
2. Leave the flow cell on the flow cell stage with the flow cell lever in position 2. The manifolds remain in the raised position.
3. [For high output modes] Load 10 ml laboratory-grade water in each reagent position in the reagent racks. Then lower the sippers.
4. [For Rapid Run mode] Load 10 ml laboratory-grade water in each reagent position in the reagent racks and 1 ml laboratory-grade water in the loading station position. Then lower the sippers.
5. Do not turn off the instrument.
6. Before using the instrument again, perform a water wash. For more information, see *Perform a Water Wash* on page 104.

Shut Down the Instrument

Shut down the instrument only if you do not plan to use it within the next 10 days or more. If you plan to use the instrument within the next 10 days, see *Idle the Instrument* on page 108.

Use the following procedure to prepare fluidics safely and shut down the system.

1. Perform a maintenance wash to flush the system. For more information, see *Perform a Maintenance Wash* on page 100.
2. Remove the flow cell from the flow cell stage.
3. Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

4. [For high output modes] Load 10 ml laboratory-grade water in each reagent position in the reagent racks. Then lower the sippers.
5. [For Rapid Run mode] Load 10 ml laboratory-grade water in each reagent position, and 1 ml laboratory-grade water in each loading station position. Then lower the sippers.
6. Turn off the instrument.
7. To restart the instrument, load water in all reagent positions, turn on the instrument, and perform a water wash. For more information, see *Perform a Water Wash* on page 104.

Real-Time Analysis

Chapter 6

Introduction

Real-Time Analysis (RTA) software performs on-instrument image analysis and base calling during a sequencing run on the HiSeq 2500, which saves valuable time during subsequent data analysis.

Real-Time Analysis Overview

Real-Time Analysis runs on the instrument computer, performing base calling and assigning a quality score to each base call.

The software tracks the status of each tile and determines when to advance it to the next process step. When advancing a tile, Real-Time Analysis outputs a file for the completed step and then starts the next step. Thus, the software can determine the status of each tile based on which files exist. If Real-Time Analysis is terminated, it saves run data and can resume processing.

Real-Time Analysis Input

Real-Time Analysis requires the following input files:

- Cluster intensity files, which contain image analysis results.
- RunInfo.xml, which the control software generates automatically at the beginning of the run. From this file, Real-Time Analysis reads the run name, number of cycles, whether a read is indexed, and the number of tiles on the flow cell.
- HiSeq.Configuration.xml, which is an instrument configuration file in XML format.
- RTA.exe.config, which is a software configuration file in XML format.

Real-Time Analysis uses run parameters entered during run setup, and receives commands from the control software that include information about when to initiate and the location of RunInfo.xml.

Real-Time Analysis Output

Tiles are small imaging areas on the flow cell defined as 1 field of view by the camera. For each tile that is analyzed, Real-Time Analysis produces a set of quality-scored base call files and filter files as primary output. Other files support generation of primary output files.

- **Base call files**—For each tile that is analyzed, 1 compressed base call (*.bcl) file is generated for each tile per cycle. The base call file contains the base call and associated quality score.
- **Filter files**—Each tile produces filter information that is included in 1 filter (*.filter) file for each tile over the whole run. The filter file specifies whether clusters pass filters.
- **Cluster location files**—One cluster location (*.locs) file contains the X,Y coordinates for every cluster on the flow cell.

- **Statistics files**—For each cycle, 1 statistics file (*.stats) is produced. The statistics file contains aggregate statistics for the cycle.

Primary output files are used for subsequent data analysis. Use bcl2fastq for demultiplexing and conversion of .bcl files into FASTQ files, which can be used as input for alignment. To convert data from the HiSeq, use bcl2fastq 1.8.4, or later.

Real-Time Analysis provides real-time metrics of run quality stored as InterOp files. InterOp files are binary files containing tile, cycle, and read-level metrics, and are required for viewing metrics in Sequencing Analysis Viewer. For viewing metrics generated by Real-Time Analysis, use Sequencing Analysis Viewer v1.8.20 or later.

For more information, see *Sequencing Output Files* on page 121

Real-Time Analysis Error Handling

Real-Time Analysis keeps log files in the RTALogs folder. If an error occurs, it is recorded in an error log file called *Error.txt.

NOTE
The software creates the error log file only if an error occurs.

Data Transfer

Throughout the run, Real-Time Analysis automatically copies data generated from raw image files to the specified output folder location. If image analysis lags, Real-Time Analysis stops processing and places the flow cell in a safe state. Processing resumes when image data are available.

NOTE
If Real-Time Analysis stops functioning, processing is automatically resumed during the next cycle at the appropriate point on the flow cell. Do not restart Real-Time Analysis manually.

If you are using BaseSpace, Illumina recommends a minimum network connection speed of 10 Mbps. For more information, see the *HiSeq 2500, 1500, and 2000 Site Prep Guide (part # 15006407)*.

Data transfer is complete when a marker file called Basecalling_Netcopy_complete.txt is generated. One of these files is generated for each read, and 1 is generated for the entire run.

Monitor Run Metrics

Real-Time Analysis automatically generates quality metrics when image analysis begins. However, not all metrics are available at the early cycles because some processes need multiple cycles to generate data.

Data	Cycle
Image analysis	After cycle 5. During the first 5 cycles of the run, the software generates a template of cluster locations.
Base calls	After cycle 12. Base calling begins after the color matrix is estimated at cycle 12.
Phasing estimates	After cycle 25. The phasing corrections for the first 25 cycles determine the phasing estimate.
Quality scores	After cycle 25. A quality score is generated for reads that pass the quality filter. Because quality scores require corrected intensities from future cycles, quality scoring always follows base calling.
Error rates	After cycle 25. Error rates are generated only when PhiX clusters are present and the **Align to PhiX** option is selected during run setup.
In-line controls	At cycle 52 of each read, or at the end of the run for runs with fewer than 52 cycles. In-line controls are generated only for TruSeq library preparation methods.*
Index count	After the index reads are complete. The index count per lane is generated only when a sample sheet is provided.

*Sequencing Analysis Viewer v1.8.44 and later no longer includes the TruSeq Controls tab, where SAV reports the results of the analysis of the in-line controls.

Sequencing Analysis Viewer

The Sequencing Analysis Viewer software shows metrics generated during the sequencing run. Metrics appear in the form of plots, graphs, and tables. Sequencing Analysis Viewer opens automatically after run metrics are available.

Select **Refresh** at any time during the run to view updated metrics.

For more information, see the *Sequencing Analysis Viewer User Guide (part # 15020619)*.

Real-Time Analysis Workflow

Real-Time Analysis and the control software perform the Real-Time Analysis workflow. The workflow includes the following steps:

- **Template generation**—Maps cluster locations.
- **Registration and intensity extraction**—Records the location of each image on the flow cell and determines an intensity value for each cluster.
- **Color matrix correction**—Corrects cross talk between channels.
- **Empirical phasing correction**—Corrects the effects of phasing and prephasing.
- **Base calling**—Determines a base call for every cluster.
- **Quality scoring**—Assigns a quality score to every base call.

Template Generation

The first step of the workflow is template generation, which defines the position of each cluster in a tile using X and Y coordinates. The template is used as a reference for the subsequent step of registration and intensity extraction.

Because of the random array of clusters on the flow cell, template generation requires image data from the first 5 cycles of the run. After the last template cycle for a tile is imaged, the template is generated.

Cluster positions are written to 1 cluster location (*.locs) file or compressed cluster location (*.clocs) file for each tile. For more information, see *Sequencing Output Files* on page 121.

Registration and Intensity Extraction

Registration and intensity extraction begin after the template of cluster positions is generated.

- Registration aligns the images produced over every cycle subsequent to template generation against the template.
- Intensity extraction determines an intensity value for each cluster in the template for a given image.

If registration fails for any images in a cycle, no base calls are generated for that tile in that cycle. Use the Sequencing Analysis Viewer to examine thumbnail images and identify images that failed registration. Use the offsets files for troubleshooting registration issues. For more information, see *Sequencing Output Files* on page 121.

Color Matrix Correction

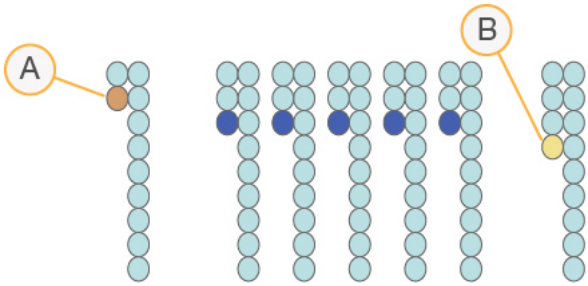
After registration and intensity extraction, Real-Time Analysis corrects for cross talk between channels. Cross talk occurs when a cluster shows intensity in the C channel and some intensity also shows in the A channel, for example. Using a 4 x 4 color matrix, Real-Time Analysis generates matrix-corrected intensities with reduced or no cross talk, and balances differences in overall intensity between color channels.

Empirical Phasing Correction

During the sequencing reaction, each DNA strand in a cluster extends by 1 base per cycle. Phasing and prephasing occurs when a strand becomes out of phase with the current incorporation cycle.

- Phasing occurs when a base falls behind.
- Prephasing occurs when a base jumps ahead.

Figure 50 Phasing and Prephasing



A Read with a base that is phasing
B Read with a base that is prephasing

Real-Time Analysis corrects the effects of phasing and prephasing using the empirical phasing correction algorithm, which maximizes the data quality at every cycle throughout the run.

Phasing and prephasing results are recorded in the file named EmpiricalPhasing_[lane]_[read]_[tile].txt, which is located in the folder Data\Intensities\BaseCalls\Phasing.

Base Calling

After raw intensities have been color corrected and phasing and prephasing corrected, the color channel with the brightest intensity is the base call for that cluster in that cycle. Base calling on the HiSeq 2500 using Real-Time Analysis begins after cycle 12.

Base calling determines a base (A, C, G, or T) for every cluster of a given tile at a specific cycle. Base calls are saved to base call (*.bcl) files, which are binary files with 1 byte per call and quality score. Each base call file contains the base call and the base call quality score. To make a base call, clusters must first pass the chastity filter. Clusters that do not pass filter or cannot be called because they are off-image or failed image registration are labeled no-calls. No-calls are represented as (N).

Clusters Passing Filter

During the first 25 cycles of Read 1, the chastity filter removes the least reliable clusters from analysis results. Clusters pass filter if no more than 2 base calls have a chastity value below 0.6 in the first 25 cycles. Chastity is the ratio of the brightest base intensity divided by the sum of the brightest and the second brightest base intensities. The percentage of clusters passing filter is represented in analysis reports as %PF.

Clusters are formed in a random array and located during template generation. Low quality clusters are removed from the raw cluster count during template generation, which yields a relatively high percentage of clusters passing filter.

Quality Scoring

A quality score, or Q-score, is a prediction of the probability of an incorrect base call. A higher Q-score implies that a base call is more reliable and more likely to be correct.

The Q-score serves as a compact way to communicate small error probabilities. Quality scores are represented as Q(X), where X is the score. The following table shows the relationship between the quality score and error probability.

Q-Score Q(X)	Error Probability
Q40	0.0001 (1 in 10,000)
Q30	0.001 (1 in 1,000)
Q20	0.01 (1 in 100)
Q10	0.1 (1 in 10)

NOTE
Quality scoring is based on a modified version of the Phred algorithm. For more information, see en.wikipedia.org/wiki/Phred_quality_score.

Quality scoring calculates a set of predictors for each base call, and then uses the predictor values to look up the Q-score in a quality table. Quality tables are created to provide optimally accurate quality predictions for runs generated by a specific configuration of sequencing platform and version of chemistry.

After the Q-score is determined, results are recorded in base call (*.bcl) files. For more information, see *Sequencing Output Files* on page 121.

Q-Score Binning

Real-Time Analysis groups quality scores into specific ranges, or bins, and assigns a value to each range. Q-score binning significantly reduces storage space requirements without affecting accuracy or performance of downstream applications.

Q-score binning contributes to the efficiency of analysis processes and data transfer requirements associated with the high throughput of the HiSeq 2500. The resulting *.bcl file is smaller because the compression algorithms are able to compress the file more effectively. Less data are written to the instrument computer and transferred to a network location, making the file copy faster.

Sequencing Output Files

File Type	File Description, Location, and Name
Base call files	Each tile analyzed is included in a base call file that contains the base call and encoded quality score. Data\Intensities\BaseCalls\L00[X]—Files are stored in per cycle folders for each lane. s_[Lane]_[Tile].bcl.gz, where lane is the single-digit lane number and tile is the 4-digit tile number. Base call files are compressed using gzip compression.
Cluster location files	For each tile, 1 cluster location file contains the XY coordinates for every cluster. Cluster location files are the result of template generation. Data\Intensities
Filter files	The filter file specifies whether a cluster passed filters. Filter files are generated at cycle 26 using 25 cycles of data. Data\Intensities\BaseCalls\L00[X]—Files are stored in 1 folder for each lane and tile. s_[lane]_[tile].filter
InterOp files	Binary reporting files used for Sequencing Analysis Viewer. InterOp files are updated throughout the run. InterOp folder
Log files	Record events and are updated throughout the run. Data\RTALogs
Offsets files	Two offsets files are created for each run: • offsets.txt—Contains tile offsets for every cycle and channel relative to the template. • SubTileOffsets.txt—Contains the measured shift for each quadrant of each image relative to the frame of reference. Data\Intensities\Offsets

File Type	File Description, Location, and Name
Phasing files	Contains empirical phasing information by tile. Phasing files are created at the first cycle base called and updated after each cycle base called. Data\Intensities\BaseCalls\Phasing EmpiricalPhasing_[lane]_[read]_[tile].txt—Tile is represented with a 4-digit number that indicates surface, swath, and tile.
Real-Time Analysis configuration file	Created at the beginning of the run, the Real-Time Analysis configuration file lists settings for the run. Data\Intensities RTAConfiguration.xml
Statistics files	Statistics created at base calling for each cycle. Data\Intensities\Basecalls\L00[X]\C[X.1]—Files are stored in 1 folder for each lane and 1 subfolder for each cycle.
Run information file	Lists the run name, number of cycles in each read, whether the read is an indexed read, and the number of swaths and tiles on the flow cell. The run info file is created at the beginning of the run. [Root folder] RunInfo.xml
Thumbnail files	A thumbnail image for each channel and tile in each swath at every cycle during imaging. Thumbnail_Images\L00[X]\C[X.1]—Files are stored in 1 folder for each lane and 1 subfolder for each cycle. s_[lane]_[tile]_[channel].jpg—Tile is represented with a 4-digit number that indicates surface, swath, and tile. See *Tile Numbering* on page 125.

Output Folder Structure

- **Config**—Configuration settings for the run.
- **Data**
  - **Intensities**
    - **BaseCalls**
      - **L00[X]**—Base call files for each lane, aggregated in 1 file per cycle.
      - **Phasing**—Empirical phasing files, 1 file per tile at every cycle.
    - **L00[X]**—Aggregated cluster location files for each lane.
    - **Offsets**—Two offsets file for the run.
    - RTAConfiguration.xml
- **Images**
  - **Focus**
    - **L00[X]**—Focus images for each lane.
- **InterOp**—Binary files used by Sequencing Analysis Viewer.
- **Logs**—Log files describing operational events.
- **Recipe**—Run-specific recipe file named with reagent cartridge ID.
- **RTALogs**—Log files describing Real-Time Analysis events.
- **Thumbnail_Images**—Thumbnail images of 9 locations from each tile, generated for each cycle and base.
- RunInfo.xml
- RunParameters.xml

Run Folder Name and Path

The run folder is the root folder for output from a sequencing run. During run setup, the software prompts you to enter the path for the run folder. By default, the folder is named in the following format:

YYMMDD_<Computer Name>_<Run Number>_<Flow Cell ID>
Example: 110114_SN106_0716_A90095ACXX

The run number increments by 1 each time you perform a run on the instrument. The flow cell ID entered during the run setup steps appends to the run folder name.

The run folder is written to the output path specified on the Scan screen during run setup. The temporary run folder for flow cell A is written to the D: drive and the temporary run folder for flow cell B is written to the E: drive.

Tile Numbering

The HiSeq high output flow cell is imaged in 96 tiles on each lane, top and bottom, for each cycle. Each lane has 3 swaths with 16 tiles per swath. The rapid flow cell is imaged in 64 tiles. Each lane has 2 swaths with 16 tiles per swath.

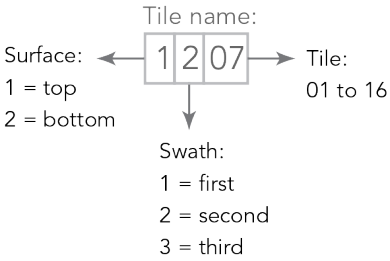
The tile name is a 4-digit number that represents the position on the flow cell.

- The first digit represents the surface:
  - 1 is for top
  - 2 is for bottom
- The second digit represents the swath:
  - 1 is for the first swath
  - 2 is for the second swath
  - 3 is for the third swath (if applicable)

NOTE
A swath is a column of tiles within a lane of the flow cell.

- The last 2 digits represent the tile, 01 through 16. Tile numbering starts with 01 at the output end of the flow cell through 16 at the input end.

Figure 51 Tile Numbering



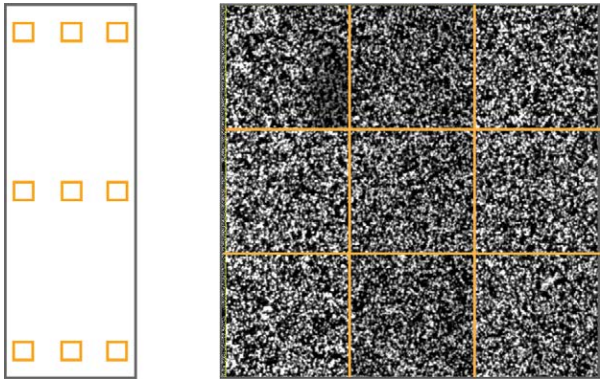
This example indicates a tile from the top surface of the flow cell, the second swath, and the seventh tile.

Thumbnail Images

You can configure the control software to generate thumbnail images in *.jpg file format. Thumbnail images are generated for each cycle and base.

The control software collects images from 9 sections of a tile. The 9 images are combined into 1 thumbnail image and can be used to troubleshoot a run. Thumbnail images are not suitable for image analysis, but can be used for troubleshooting.

Figure 52 Thumbnail Image



Troubleshooting

Chapter 7

Introduction

This section describes what to do if a problem occurs during a sequencing run and how to perform a fluidics check from the Welcome screen.

Possible Run Setup Problems

Problem	Possible Cause	Action
The software did not initialize.	The software was unable to initialize internal hardware devices.	Close the error message and then relaunch the instrument software. If the problem persists, restart the instrument computer. If you are going to restart the computer, first shut down the instrument to make sure the DoNotEject drive is recognized correctly. If the problem persists after restarting the instrument computer, shut down the instrument, wait a minimum of 60 seconds, and then restart the instrument.
Flow cell lever is orange.	The flow cell did not seat properly. The vacuum did not seal. Manifolds did not raise.	Remove the flow cell and repeat the cleaning steps. Make sure that the gaskets are present and well-seated. Reload the flow cell. If the preceding steps do not work, try replacing the gaskets, and then reload the flow cell.
Flow cell lever is blinking orange.	Vacuum is being provided but is inadequate.	Remove the flow cell and repeat the cleaning steps. Make sure that the gaskets are present and well-seated. Reload the flow cell. If the preceding steps do not work, try replacing the gaskets, and then reload the flow cell.
Flow cell lever is blinking green.	Vacuum pressure is good.	Switch flow cell lever to position 2.
Poor fluid delivery.	Potential bubbles in the system.	Reposition the flow cell and confirm that the holes are facing ***down***. Look for white precipitate around the gaskets. If precipitate is present, replace the gaskets. Always replace gaskets before an instrument maintenance wash. Confirm that the sipper assemblies are fully lowered and each sipper is in contact with the reagents.

Stagger Runs on Flow Cell A and Flow Cell B

1 Wait for the run on the adjacent flow cell to begin a chemistry step, and then select **Pause**. The Pause menu opens.

NOTE
Always pause the current run during a chemistry step opposed to an imaging step.

2 Select **Normal Pause**.

3 Wait for the software to complete the current chemistry step. The system is placed in a safe state automatically.

4 After the adjacent run is paused, set up the new run.

5 After loading the new flow cell for the new run, close the compartment door.

6 Select **Start** to start the new sequencing run.

7 Select **Resume** on the adjacent flow cell to resume the paused run. The software automatically controls chemistry and imaging processes on both flow cells.

Perform a Fluidics Check

The Check button on the Welcome screen performs a fluidics check. Use this option during instrument installation and when troubleshooting fluidics issues.

1. Load a used flow cell onto the instrument.
2. Load 8 SBS bottles with PW1 or laboratory-grade water, and load the bottles onto the corresponding reagent rack. Load the rack onto the instrument.
3. Select **Check** on the Welcome screen.
4. Select solution 5 (SB2) from the drop-down list. If you are performing a fluidics check with a used flow cell, select solution 2, which is water.
5. Enter the following default values:
   - Volume: **250**
   - [For HiSeq v4 and TruSeq v3 modes] Aspirate Rate: **250**
   - [For Rapid Run mode] Aspirate Rate: **1500**
   - Dispense Rate: **2000**
6. Select **Pump**. To pause the fluidics check, select **Pause**.
7. Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
   If excessive bubbles are present, check the manifold gaskets for obstructions, reduce the aspirate rate to 100, and pump another 250 µl of water to the flow cell.

BaseSpace is Unavailable

If BaseSpace is not available, open Windows Services to make sure that the BaseSpace Broker has started. If it has not started, restart it. If services are running and BaseSpace is still unavailable, contact Illumina Technical Support.

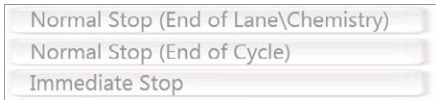
Stop and Resume a Run

Stopping a run might be necessary if the run was set up incorrectly, if the data quality is bad, or if there is a hardware error. To resume a stopped run, make sure that you select the appropriate normal stop options that allow the run to resume.

Stop Option	Real-Time Analysis Option	Able to Resume?
Normal Stop (End of Lane\Chemistry)	Keep As Is	Yes. The run resumes at the next chemistry or imaging command.
	Complete For Run	No. The run cannot be resumed.
	Complete For Read	Yes. The run resumes at the beginning of the next read.
Normal Stop (End of Cycle)	Keep As Is	Yes. The run resumes at the next cycle.
	Complete For Run	No. The run cannot be resumed.
	Complete For Read	Yes. The run resumes at the beginning of the next read.
Immediate Stop	No option	No.

1 From the Run Overview screen, select **Stop**. The stop menu opens.

Figure 53 Stop the Run



2 Select 1 of the following stop options:

3 Select a stop option:

- **Normal Stop (End of Lane\Chemistry)**—Stops the run only after the current chemistry or imaging command is complete, and then places the flow cell in a safe state.
- **Normal Stop (End of Cycle)**—Stops the run after the current cycle is complete, and then places the flow cell in a safe state.
- **Immediate Stop**—Stops the run without completing the current operation and does ***not*** place the flow cell in safe state. You cannot resume a run that was stopped using the immediate stop option.

4 Select from the following Real-Time Analysis options:

- **Keep As Is**—The run is stopped without any modifications to Real-Time Analysis. The run can resume where it was stopped.
- **Complete For Run**—Real-Time Analysis is stopped. The run info, run parameters, and recipe files are updated to reflect total cycles as the last cycle completed. Then Real-Time Analysis restarts to complete base calling for the run up to the point the run was stopped. The run cannot resume.
- **Complete For Read**—Real-Time Analysis is stopped. The run info, run parameters, and recipe files are updated to trim the length of the current read to the last cycle completed. Subsequent reads are not affected. Then Real-Time Analysis restarts to complete analysis for the current read. The run can resume at the beginning of the next read.

5 After the run is stopped, select **Return to Start** on the Run Overview screen. The Welcome screen opens.

Resume a Stopped Run

A run can be resumed only when the run was stopped safely using a normal stop option with a Real-Time Analysis option that allows the run to resume.

NOTE
If the adjacent side is performing cluster generation or paired-end chemistry, the run does not resume until the ongoing process is complete.

1 From the Welcome screen, select **Sequence**, and then select **Resume Run**. The Resume screen opens.

2 Select the appropriate run folder from the drop-down list.

NOTE
The software resumes the run at the point where the run was stopped, and defaults to the correct setup on the Resume screen.

3 Confirm the settings on the Resume screen or select the appropriate point in the run to resume the run.

- **Resume At** lists the read or point in the run to resume.
- **Start At Cycle** lists the cycle to resume.

CAUTION
Illumina does not recommend resuming a run at the point of paired-end turnaround, other than for Read 2 primer rehybridization.

4 Confirm the settings on the Resume screen or select the imaging and chemistry commands to resume. For more information, see *Example Settings for Resuming a Run* on page 135.

5 Select **Next** to proceed. The software guides you through the remaining run setup steps.

Example Settings for Resuming a Run

If the run was stopped after imaging lane 1 at cycle 23, the software automatically sets up the resume run settings for Read 1 at cycle 23. The system shows the following settings on the Resume screen:

- **Resume At:** Read 1
- **Start At Cycle:** 23

Figure 54 Example of Resume at Cycle 23

Because the run in this example was stopped during an imaging step, **Imaging (no chemistry)** is selected automatically.

Pause a Run

CAUTION
Do not pause a run during imaging. Use the Normal stop, end of cycle or end of lane feature, to stop and resume a run.

Pause a run from the Run Overview screen. Pausing a run might be necessary to check run components, such as reagent volumes, before proceeding with the run. Under normal operation, pausing a run is not necessary.

1 From the Run Overview screen, select **Pause**. The pause menu opens.

Figure 55 Pause Options

2 Select **Normal Pause**.

3 Select **Yes** to confirm the pause command. The software completes the current chemistry or imaging command and places the flow cell in a safe state.

4 Select **Resume** to resume the run.

Change Reagents During a Run

If you started the run with a partial volume of reagents, use the Change Reagents feature to pause the run and replenish reagents.

1 From the Run Overview screen, select **Pause**. The pause menu opens.

2 Select **Change Reagents**.

3 Select **Yes** to confirm the pause command. The software completes the current chemistry or imaging command, places the flow cell in a safe state, and opens the Reagents screen.

4 On the Reagents screen, enter the following reagent parameters:
- The reagent kit ID for new reagents.
- The number of cycles the reagents are expected to last.

NOTE
The priming checkbox is disabled on the Change Reagents screen. Priming is not required.

5 Select **Next** to proceed to loading reagents.

Primer Rehybridization

A rehybridization run repeats the sequencing primer hybridization step. If run metrics indicate low cluster numbers, low cluster intensities, or other concerns, perform primer rehybridization to rescue the flow cell. Primer rehybridization does not damage clusters on the flow cell.

HiSeq v4 Flow Cell

All rehyb steps are performed on the HiSeq 2500. The kit includes primers for Read 1, Index 1 Read, Index 2 Read for single-read flow cells, and Read 2.

Rehyb Kit Name	Workflow Instructions
HiSeq Multi-Primer Rehyb Kit v4 Catalog # GD-403-4001	*HiSeq Primer Rehybridization Guide (part # 15050105)*

HiSeq v3 Flow Cell

Read 1 primer rehyb is performed on the cBot. The kit includes a cBot reagent plate containing the Read 1 sequencing primer HP6. For Nextera libraries, use HP10 from the TruSeq Dual Index Sequencing Primer Box.

Rehyb Kit Name	Workflow Instructions
TruSeq v2 cBot Multi-Primer Rehyb Kit Catalog # GD-304-2001	*Read 1 Primer Rehybridization on the cBot (part # 15018149)*

Rapid Flow Cell

All rehyb steps are performed on the HiSeq 2500. The kit includes primers for Read 1, Index 1 Read, Index 2 Read for single-read flow cells, and Read 2.

Rehyb Kit Name	Workflow Instructions
HiSeq Rapid Rehyb Kit Catalog # GD-404-1001	*HiSeq Rapid Run Primer Rehybridization (part # 15059379)*
TruSeq Rapid Rehyb Kit Catalog # GD-402-2001	*TruSeq Rapid Run Primer Rehybridization (part # 15039627)*

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 16 Illumina General Contact Information

Address	5200 Illumina Way San Diego, CA 92122 USA
Website	www.illumina.com
Email	techsupport@illumina.com

Table 17 Illumina Customer Support Telephone Numbers

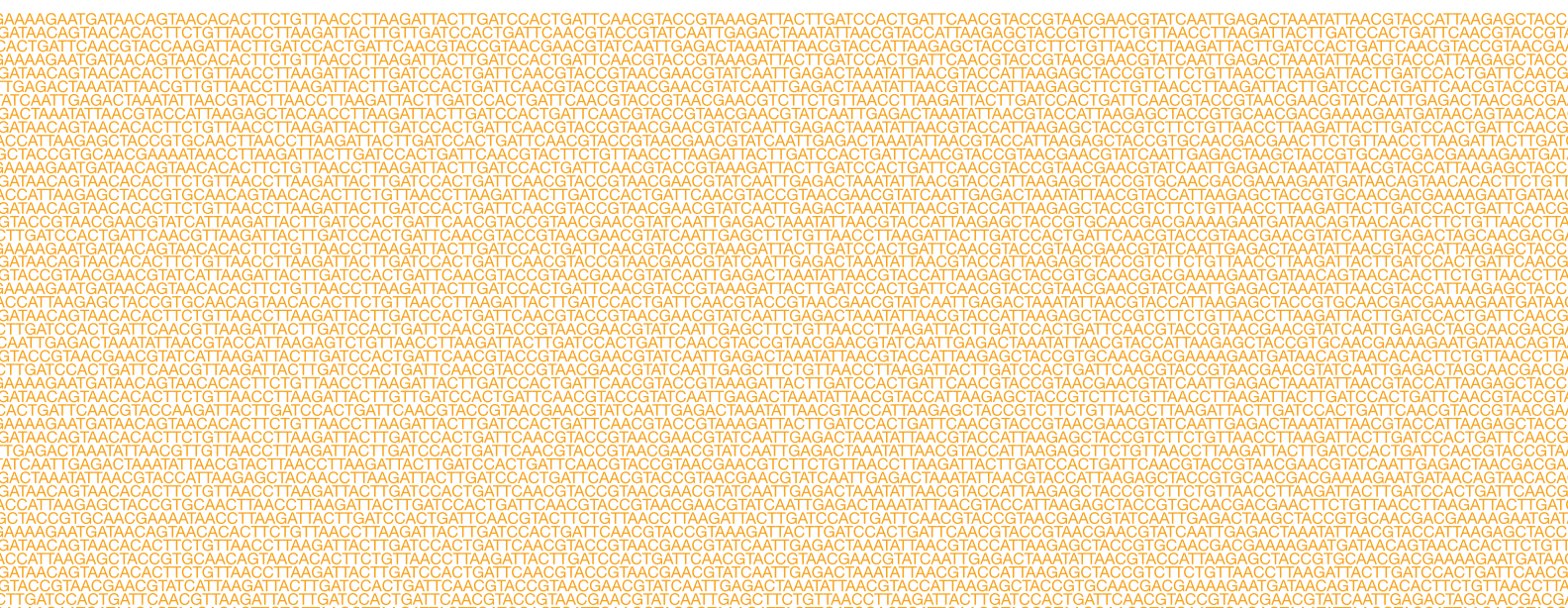
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then click **Documentation & Literature**.



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